The Correlation of Penicillinase Production and Bacteriophage Adsorption of *Staphylococcus aureus*

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INTRODUCTION

One of the very common organisms which displays degrees of antibiotic resistance is Staphylococcus aureus, the organism selected for this study. Staphylococcus aureus has a very wide distribution in nature, as it is found on the skin surface, in nasal passages, and in a variety of food products. Staphylococci may be the causative agents of boils, furuncles, osteomyelitis, secondary meningitis, septicemia, food poisoning and pneumonia.

The virulence of Staphylococcus aureus is primarily due to the production of metabolic products which have serious effects on physiological activities of the host. Perhaps the most reliable indication of staphylococcus pathogenicity is the production of a procoagulase which unites with a factor in citrated or oxalated blood plasma to coagulate fibrinogen, causing fibrin to be deposited on the bacterial cell walls which protects them from phagocytosis.

One product of staphylococcus metabolism, hyaluronidase, hydrolyzes hyaluronic acid, a tissue binding component in living cells, and facilitates the spreading of the infection.

ASSOCIATED WITH MANY TYPES OF BACTERIA ARE BACTERIOPHAGES, VIRUS-LIKE PARTICLES WHICH INFECT THE SPECIFIC BACTERIUM. THE PHAGES ARE CLASSIFIED ACCORDING TO THEIR REACTION INSIDE THE BACTERIA. LYTIC BACTERIOPHAGES CAUSE LYSIS OF THE BACTERIAL CELL FOLLOWING PHAGE INFECTION. LYSOGENIC PHAGES, AFTER PENETRATION OF THE HOST CELL INTERIOR, DO NOT MULTIPLY AND BECOME AN INTEGRAL PART OF THE GENETIC APPARATUS OF THE BACTERIAL CELL. THE PHENOMENON OF LYSOGENY HAS SERVED AS A POWERFUL TOOL IN STUDIES OF GENETIC STRUCTURES. LYTIC PHAGES HAVE ALSO PLAYED A LEADING ROLE IN BACTERIAL GENETICS THROUGH THE PHENOMENON OR TRANSDUCTION.

IN ELECTRON MICROPHOTOGRAPHS, STAPHYLOCOCCAL PHAGES RESEMBLE TADPOLES WITH AN AVERAGE SIZE HEAD OF 55 MILLIMICRONS AND TAIL OF 225 MILLIMICRONS IN LENGTH. THE FEW ANALYSES AVAILABLE ON THE GROSS CHEMICAL COMPOSITION OF PHAGES SEEM TO BE IN GENERAL AGREEMENT THAT THESE PHAGES ARE ABOUT HALF PROTEIN AND HALF DEOXYRIBOSE NUCLEIC ACID. THE WELL-KNOWN PLANT VIRUSES CONTAIN RIBONUCLEIC ACID (RNA) EXCLUSIVELY, USUALLY IN RELATIVELY SMALL AMOUNTS. MOST ANIMAL VIRUSES ARE COMPOSED OF RNA AND SMALLER AMOUNTS OF DEOXYRIBOSE NUCLEIC ACID.

THE LATTER INCLUDE THE CAREFULLY STUDIED PR8 AND LEE STRAINS OF INFLUENZA VIRUS WHICH ARE REPORTED AS HAVING 0.9 PER CENT RNA AND 0.1 PER CENT DNA, AND MEF-1 POLIOMYELITIS VIRUS WHICH ANALYZED 24 PER CENT RNA AND 1 PER CENT DNA. DNA HAS ALSO BEEN REPORTED IN SOME INSECT VIRUSES.

WHEN INFECTED WITH A TEMPERATE PHAGE, A BACTERIUM MAY RESPOND IN AT LEAST TWO DIFFERENT WAYS. THE INFECTING PHAGE MAY ENTER THE VEGETATIVE STATE, REPRODUCE, AND THE BACTERIA WILL LYSE. THE OTHER RESPONSE INVOLVES THE PHAGE INFECTION BUT NO LYSIS. THE FREQUENCY OF THESE RESPONSES DEPENDS ON THE CONDITIONS OF INFECTION AND THE GENETIC CONSTITUTION OF THE PHAGE. TEMPERATURE AND THE NUMBER OF PHAGE PER BACTERIUM ARE TWO CONDITIONS WHICH MAY INFLUENCE THE BACTERIAL RESPONSE TO LYSOGENY. HOWEVER, THE CAPACITY OF THE PHAGE TO LYSOGENIZE IS COMPLETELY UNDER GENETIC CONTROL.

COAGULASE-POSITIVE STAPHYLOCOCCI ARE TYPED BY OBSERVING THE PATTERN REACTIONS WHEN EXPOSED TO DIFFERENT PHAGES. WIDESPREAD LYSOGENICITY HAS BEEN OBSERVED IN STAPHYLOCOCCAL PHAGES. PHAGE TYPING OF THE STAPHYLOCOCCI PRESENTS MORE PROBLEMS THAN TYPING THE SALMONELLAS OR OTHER PATHOGENIC ENTEROBACTERIACEAE. THE STAPHYLOCOCCI

ARE MUCH MORE WIDE SPREAD THAN THE ENTERIC PATHOGENS; THEREFORE, A MUCH GREATER VARIETY OF TYPES MIGHT BE EXPECTED. HENCE, THE USE OF TYPE DESIGNATIONS IS IMPRACTICAL. HOWEVER, IN TRACING INFECTIONS IN HOSPITALS OR LOCALIZED OUTBREAKS, PHAGE-TYPING IS RELATIVELY VALUABLE.

STAGES IN PHAGE MULTIPLICATION ARE INITIATED WITH ADSORBTION OF THE PHAGE PARTICLE AND PENETRATION OF THE CELL WALL. THIS IS FOLLOWED BY THE INJECTION OF VIRAL DNA INTO THE CELL. COMPLETED PHAGE PARTICLES DO NOT REAPPEAR UNTIL THE MIDPOINT OF THE LATENT PERIOD. MORPHOLOGICALLY RECOGNIZABLE ELEMENTS ARE FORMED EARLIER WHICH CORRESPOND TO THE PROTEIN OUTER LAYER OF THE PHAGE.

THE SITES AT WHICH BACTERIA ADSORB THE PHAGE PARTICLES ARE REFERRED TO AS BACTERIAL RECEPTORS. AS A RULE, ONLY BACTERIA THAT CAN BE INFECTED BY A PHAGE CAN ADSORB IT. HOWEVER, CASES HAVE BEEN REPORTED IN WHICH PHAGE CAN BE ADSORBED BY BACTERIA UNABLE TO SUPPORT ITS GROWTH WHICH ARE THEN LYSED; SEROLOGICAL CROSS-REACTIONS HAVE BEEN FOUND BETWEEN THE NORMAL HOST FOR THE PHAGE AND THE NON-HOST BACTERIA THAT CAN ADSORB THE PHAGE. A FAIRLY GOOD CORRELATION EXISTS BETWEEN THE POSSESSION OF CERTAIN ANTIGENS AND THE SENSITIVITY TO DIFFERENT

BACTERIOPHAGES.

THE PHAGES ADSORB BY THE TIPS OF THEIR TAILS TO THE SPECIFIC RECEPTOR SITES ON THE HOST CELL SURFACE. ADSORPTION PROBABLY INVOLVES AT LEAST TWO SUCCESSIVE STEPS, THE FIRST OF WHICH IS REVERSIBLE. IN SOME CASES THE FORMATION OF SALT LINKAGES BETWEEN CARBOXYL AND AMINO GROUPS IS INVOLVED, WHILE IN OTHER CASES A MAJOR BACTERIAL ANTIGEN IS THE PHAGE RECEPTOR SUBSTANCE.

MANY OF THE STAPHYLOCOCCI STRAINS WHICH ARE ASSOCIATED WITH EPIDEMICS ARE ANTIGENICALLY RELATED SINCE MANY OF THEM ARE INFECTED BY THE SAME PHAGES. THIS OBSERVATION SUGGESTS THE HYPOTHESIS THAT ANTIBIOTIC RESISTANCE MAY BE RELATED TO PHAGE ADSORPTION.

PENICILLIN ACTIVITY ON BACTERIAL CELLS HAS BEEN DESCRIBED AS AN INHIBITION OF CELL WALL SYNTHESIS BY PREVENTING THE POLYMERIZATION OF AN ESSENTIAL CELL WALL CONSTITUENT. ACCORDING TO PARK AND STROMINGER, THERE ARE SUBSTANCES IN THE CELL WALL WHICH ARE UNIQUELY SIMILAR TO A PRODUCT WHICH ACCUMULATES INTRACELLULARLY IN ANTIBIOTIC-TREATED STAPHYLOCOCCI. MANY EXPERIMENTS POINT TO THE CONCLUSION THAT URIDINE PYROPHOSPHATE N-ACETYL-AMINO SUGAR IS THE BIOSYNTHETIC PRECURSOR OF THE BACTERIAL CELL WALL, AND THAT THE ACCUMULATION OF

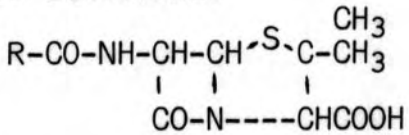
THIS COMPOUND IN PENICILLIN-TREATED STAPHYLOCOCCI IS IS THE CONSEQUENCE OF INTERFERENCE BY PENICILLIN WITH THE COMPLETION OF THE BIOSYNTHESIS OF THE CELL WALL. URIDINE PYROPHOSPHATE GLYCOSYL COMPOUNDS ARE ACTIVATED INTERMEDIATES IN MANY BIOSYNTHETIC TRANSGLYCOSIDATION REACTIONS AND THE N-ACETYL-AMINO SUGAR PEPTIDE MAY BE CONSIDERED A NUCLEOTIDYL FRAGMENT ACTIVATED BY SUCH A SYNTHETIC REACTION. THE TRANSFER OF THIS FRAGMENT FROM THE NUCLEOTIDE TO SOME CELL WALL ACCEPTOR WOULD BE A REACTION FOR WHICH MANY MODELS NOW EXIST.

THE EXACT NATURE OF THE INTERFERENCE IS A MATTER OF SPECULATION, BUT IT SEEMS POSSIBLE THAT PENICILLIN IS A SPECIFIC INHIBITOR OF THE TRANSGLYCOSIDATION REACTION INVOLVING THIS URIDINE NUCLEOTIDE. EXTENSIVE INVESTIGATION ON THE BINDING OF PENICILLIN ARE CONSISTENT WITH THIS FORMULATION. IT HAS BEEN SHOWN THAT THE PARTICLES THAT BIND PENICILLIN WERE ORIGINALLY PORTIONS OF THE CELL MEMBRANE. IF THE HYPOTHETICAL TRANSGLYCOSIDASE, IS ALSO THE SPECIFIC BINDING SITE OF PENICILLIN, IT IS STRATEGICALLY LOCATED TO TRANSFER THE N-ACETYL-AMINO SUGAR PEPTIDE FROM THE URIDINE PYROPHOSPHATE, WHICH IS INSIDE THE MEMBRANE, TO AN ACCEPTOR OUTSIDE THE MEMBRANE.

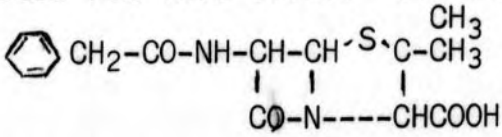
SIMILAR WORK HAS BEEN REPORTED BY LEDERBERG WITH *ESCHERICHIA COLI* LEADING TO THE CONCLUSION THAT NEW CELL-WALL FORMATION RATHER THAN EXISTING WALL STRUCTURE IS THE PROBABLE TARGET OF PENICILLIN.

ANTIBIOTIC RESISTANCE IS BELIEVED TO BE DUE TO THE PRODUCTION OF AN ENZYME WHICH UNITES WITH ITS SPECIFIC ANTIBIOTIC SUBSTRATE TO INACTIVATE THE SUBSTRATE. STAPHYLOCOCCAL PENICILLINASE HAS BEEN DEMONSTRATED TO BE AN ESSENTIALLY INTRACELLULAR ENZYME. DUE TO ITS INTRACELLULAR NATURE, ISOLATION AND PURIFICATION HAVE NOT BEEN PERFECTED.

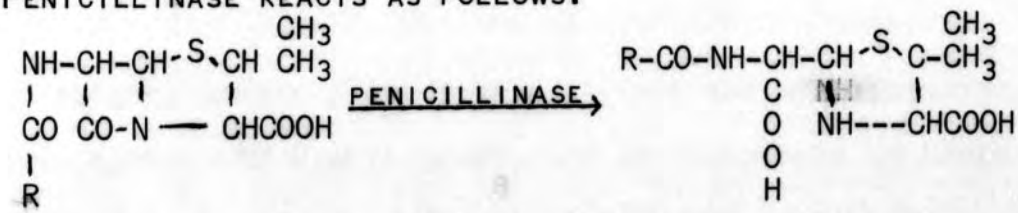
THE PENICILLINS ARE RELATIVELY UNSTABLE COMPOUNDS HAVING THE BASIC STRUCTURE:



THE R-GROUP DIFFERS WITH THE TYPE OF PENICILLIN. PENICILLIN G WAS USED FOR THIS STUDY. ITS STRUCTURE IS:



PENICILLINASE REACTS AS FOLLOWS:



THE REACTION OF PENICILLIN AND PENICILLINASE IN A BICARBONATE BUFFER RESULTS IN THE EVOLUTION OF CARBON DIOXIDE PROPORTIONAL TO THE AMOUNT OF PENICILLIN INACTIVATED DUE TO THE INTRODUCTION OF ACID INTO THE BICARBONATE SYSTEM.

PENICILLIN HAS LITTLE EFFECT ON THE PHAGE PARTICLE. THE ONLY EFFECT IS A SHORTENING OF THE LATENT PERIOD. SINCE BOTH ADSORPTION AND PENICILLIN ACTIVITY ARE CONCERNED WITH REACTIONS TAKING PLACE NEAR THE CELL SURFACE, AN ATTEMPT WAS MADE TO CORRELATE PHAGE ADSORPTION AND PENICILLINASE PRODUCTION THROUGH THIS EXPERIMENT.

MATERIALS AND METHODS

The organisms were isolated from three different patients at Moses H. Cone Memorial Hospital, Greensboro, North Carolina, and were selected for this study on the basis of the results of sensitivity to penicillin discs.

The bacteria were phage-typed by Dr. Nell Hirshberg of the U. S. Public Health Laboratory in Raleigh, North Carolina. The staphylococcus isolants will be referred to as strain I, strain II, and strain III. Strain I is lysed by phage $80^{\pm}/81$; strain II is lysed by phage 81, and strain III attacked by phages $52^{\pm}/52A^{\pm}/80^{\pm}/81$. The plus and minus denote partial and complete lysis. The common phage is type 81.

The organisms were cultured in 5 ml. of Difco Trypticase Soy broth prior to experimentation. In order to produce penicillinase the organisms must be supplied with a complex of amino acids which is furnished by this medium.

Penicillinase production is considered a mutation; therefore, strain I and strain II, the resistant strains, were exposed to minute quantities of penicillin in order to eliminate reverse mutants. Growth was initially

INHIBITED; THEREFORE, 0.05 ML. OF A 24-HOUR SUSPENSION WAS TRANSFERRED TWICE IN 48 HOURS TO INSURE A COMPARABLE NUMBER OF ORGANISMS OF EACH STRAIN AFTER A 24-HOUR GROWTH PERIOD AT 37°C.

THE STRAINS DIFFERED IN THEIR SENSITIVITY TO PENICILLIN. THE SENSITIVITY WAS DETERMINED BY THE TWO-FOLD SERIAL DILUTION TUBE METHOD WHICH GIVES A HIGHER DEGREE OF ACCURACY THAN THE DISC METHOD. FIFTY THOUSAND UNITS OF PENICILLIN WERE SERIALLY DILUTED IN 0.25 ML. OF BROTH AND 0.25 ML. OF A 1:100 DILUTION OF A FOURTEEN-HOUR SUSPENSION OF ORGANISMS WAS ADDED. THIS PREPARATION WAS INCUBATED AT 37°C FOR 24 HOURS AND THE SENSITIVITY WAS DETERMINED BY THE APPEARANCE OF GROWTH IN THE TUBE CONTAINING THE LOWEST DILUTION OF PENICILLIN. CONTROLS INOCULATED SIMULTANEOUSLY WITH THE TEST DILUTIONS WERE RUN FOR EACH DILUTION.

CRYSTALLINE POTASSIUM SALT OF PENICILLIN G (ELI LILLY CORPORATION) WHICH IS WATER-SOLUBLE WAS USED FOR THE STUDY.

PENICILLINASE ACTIVITY WAS DETERMINED BY THE AMOUNT OF CARBON DIOXIDE EVOLVED WHEN AN ALIQUOT OF AN OVERNIGHT SUSPENSION WAS EXPOSED TO PENICILLIN IN A BICARBONATE BUFFER. WITH THE WARBURG CONSTANT VOLUME RESPIROMETER,

MICROLITERS OF CO_2 EVOLVED WERE MEASURED BY THE DIRECT METHOD. A 5% KOH SOLUTION WAS PLACED IN THE CENTER WELL OF ONE VESSEL TO ABSORB THE CO_2 AND MEASURE ONLY OXYGEN UPTAKE. IN THE OTHER VESSEL, KOH WAS NOT PRESENT; THEREFORE, THE DIFFERENCE BETWEEN THE MEASUREMENTS OF THESE VESSELS DETERMINED CO_2 EVOLUTION.

A 1.0 ML. ALIQUOT OF OVERNIGHT CULTURES OF THE THREE STRAINS IN A 1.7×10^{-3} $NaHCO_3$ BUFFER (PH 7) WAS RUN SIMULTANEOUSLY AT $37^{o}C$ WITH 10,000 UNITS OF PENICILLIN IN THE SIDE ARM OF THE VESSEL. THE EQUILIBRATION PERIOD WAS 12 MINUTES AFTER WHICH 3 FIVE-MINUTE READINGS WERE TAKEN BEFORE PENICILLIN WAS DUMPED FROM THE SIDE ARM OF THE VESSEL. FIVE-MINUTE READINGS WERE TAKEN OVER A PERIOD OF 70 MINUTES.

AT THE END OF THE PERIOD, ALIQUOTS OF THE CONTENTS OF THE VESSELS WITH THE RESISTANT ORGANISMS WERE TESTED FOR ANTIBIOTIC ACTIVITY AGAINST STRAIN III. THESE ALIQUOTS WERE DILUTED 1:100 WITH A MIXTURE OF 5 ML. OF BROTH AND 95 ML. OF DISTILLED WATER AND PASSED THROUGH A SEITZ BACTERIOLOGICAL FILTER.

ISOLATION OF THE PHAGES INVOLVED SEITZ FILTERING A 24-HOUR SLANT CULTURE WHICH HAD BEEN WASHED OFF WITH 5 ML. OF BROTH. THE PHAGE IN THIS PREPARATION REMAINS

VIABLE UNDER REFRIGERATION FOR AN EXTENDED PERIOD.

PHAGE ADSORPTION WAS QUANTATIVELY MEASURED BY FOLLOWING THE DISAPPEARANCE OF FREE BACTERIOPHAGE FROM A MIXTURE OF BACTERIA. THE BACTERIAL CELLS WERE SEPARATED BY CENTRIFUGATION AND THE PHAGE WAS TITRATED FROM THE SUPERNATANT. MEASUREMENTS WERE MADE IN THE LATENT PERIOD BEFORE LYSIS. A REDUCTION OF THE TEMPERATURE PROLONGS THE LATENT PERIOD. THE ADSORPTION RATE IS INFLUENCED BY THE PHYSIOLOGICAL CONDITIONS OF THE HOST AND THE COMPOSITION OF THE MEDIUM. THE BROTH PROVIDES OPTIMAL CONDITIONS FOR ADSORPTION.

THE BACTERIAL CONCENTRATION (1.8×10^9 PER ML.) WAS DETERMINED TURBIMETRICALLY WITH A NEPHALOMETER BY CENTRIFUGING THE BROTH AND RESUSPENDING IN SALINE.

THE INITIAL CONCENTRATION OF THE PHAGE PREPARATION WAS DETERMINED SIMULTANEOUSLY WITH THE ADSORPTION MEASUREMENT IN ORDER TO HAVE THE SAME PHAGE CONCENTRATION AND BACTERIAL COUNT.

FROM THE 24-HOUR CULTURES 1 ML. OF BACTERIAL CULTURE AT 37°C WAS MIXED WITH 1 ML. OF PHAGE PREPARATION. AFTER THOROUGH MIXING, DUPLICATE 0.1 ML. SAMPLES OF THE ADSORPTION MIXTURE WERE REMOVED AFTER TWO MINUTES AND DILUTED 1:100 IN CHILLED BROTH TO STOP THE ADSORPTION

PROCESS. TWO-MILLILITER AMOUNTS OF THE CHILLED DILUTED SAMPLES WERE THEN CENTRIFUGED AT 3,000 R.P.M. FOR 5 MINUTES TO SEDIMENT THE BACTERIA AND ADSORBED PHAGE.

A 1 ML. ALIQUOT OF THE SUPERNATANT WAS THEN ASSAYED BY THE AGAR LAYER METHOD. THIS INVOLVED MIXING THE 1 ML. ALIQUOT WITH 0.05 ML. OF THE 24-HOUR BACTERIAL CULTURE IN 2.5 OF 0.75% AGAR MEDIUM AT 46°C IN ORDER TO MAINTAIN THE LIQUID STATE. THIS PREPARATION WAS POURED ON 1.5% AGAR PLATES AND ALLOWED TO INCUBATE FOR 24 HOURS. PHAGE ADSORPTION WAS THEN DETERMINED BY COMPARING THE NUMBER OF PLAQUES FORMED BY THE UNADSORBED PREPARATION AND THE ADSORBED PREPARATION.

RESULTS

By the tube sensitivity test, 0.25 ml. of 1:100 dilution of strain I was found to be sensitive to a concentration of 3,125 penicillin units. Strain II was sensitive to a concentration of 781 units. Strain III growth was inhibited by the lowest concentration of 43 units. This was in accord with observations at the hospital by the disc method.

When the aliquots of the three strains were shaken in the Warburg apparatus with penicillin in bicarbonate buffer, the output of CO_2 abruptly increased with the two resistant strains. Strain I demonstrated maximum activity in 30 minutes after penicillin exposure. Strain II showed optimal activity, evolving approximately the same amount of gas, in 25 minutes. Strain III displayed no penicillinase activity. See Figures I, II, III.

Following the 70-minute reading period, the Seitz-filtered aliquots demonstrated no penicillin activity against the sensitive strain by the tube sensitivity test.

The adsorption phase of this study introduced several unanticipated diffuculties. There was no

FIGURE I

PENICILLIN-PENICILLINASE ACTIVITY DETERMINED MANOMETRICALLY IN THE WARBURG CONSTANT VOLUME RESPIROMETER AT 37°C FOR STRAIN I STAPHYLOCOCCUS AUREUS

KEY: — μL. PER 5 MIN. INTERVAL WITH PENICILLIN
— μL. PER 5 MIN. INTERVAL WITHOUT PENICILLIN
— μL. CUMULATIVE WITH PENICILLIN
— μL. CUMULATIVE WITHOUT PENICILLIN

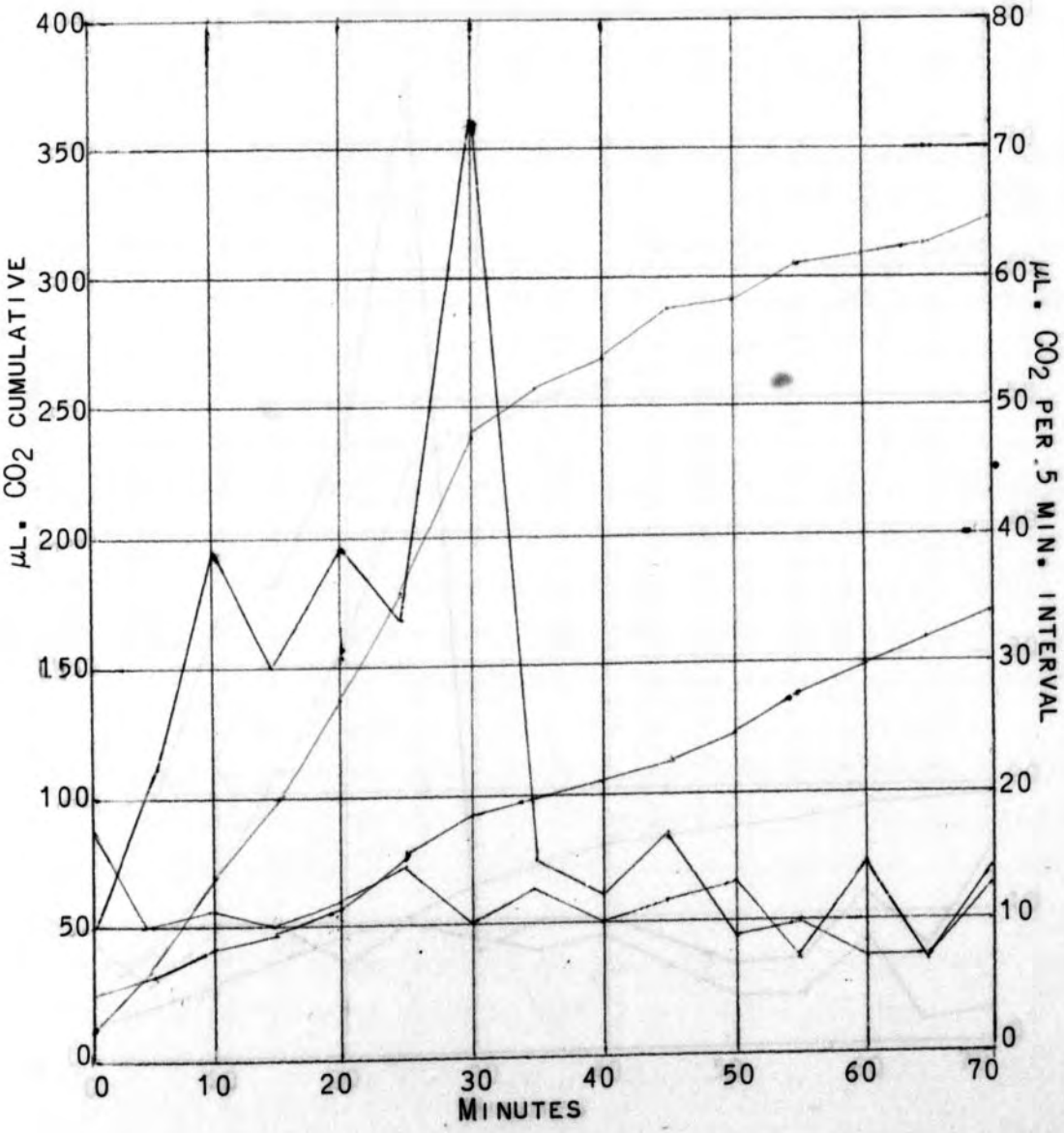


FIGURE II

PENICILLIN-PENICILLINASE ACTIVITY DETERMINED MANOMETRICALLY IN THE WARBURG CONSTANT VOLUME RESPIROMETER AT 37°C FOR STRAIN II STAPHYLOCOCCUS AUREUS

KEY: — μL. PER 5 MIN. INTERVAL WITH PENICILLIN
— μL. PER 5 MIN. INTERVAL WITHOUT PENICILLIN
— μL. CUMULATIVE WITH PENICILLIN
— μL. CUMULATIVE WITHOUT PENICILLIN

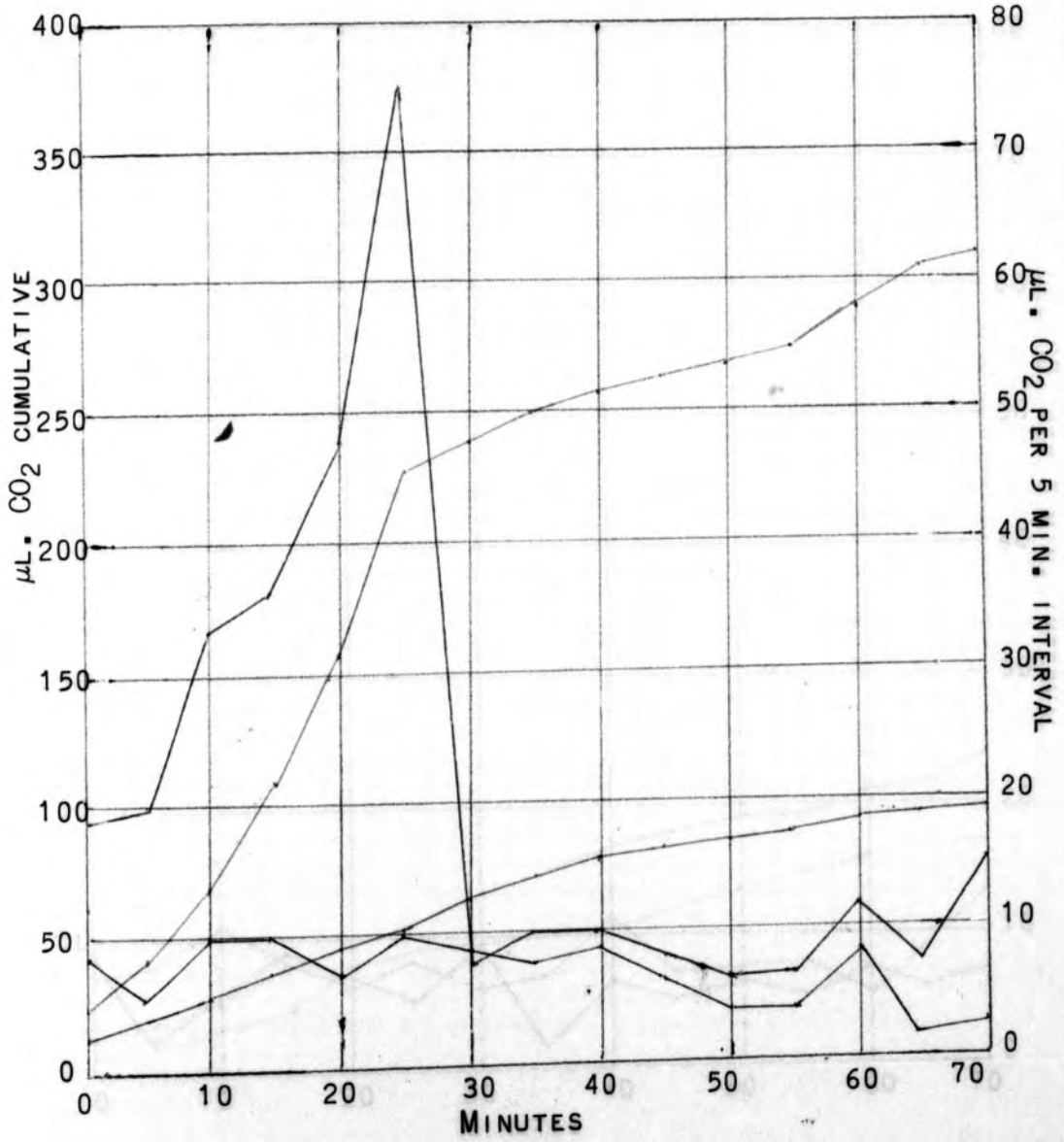
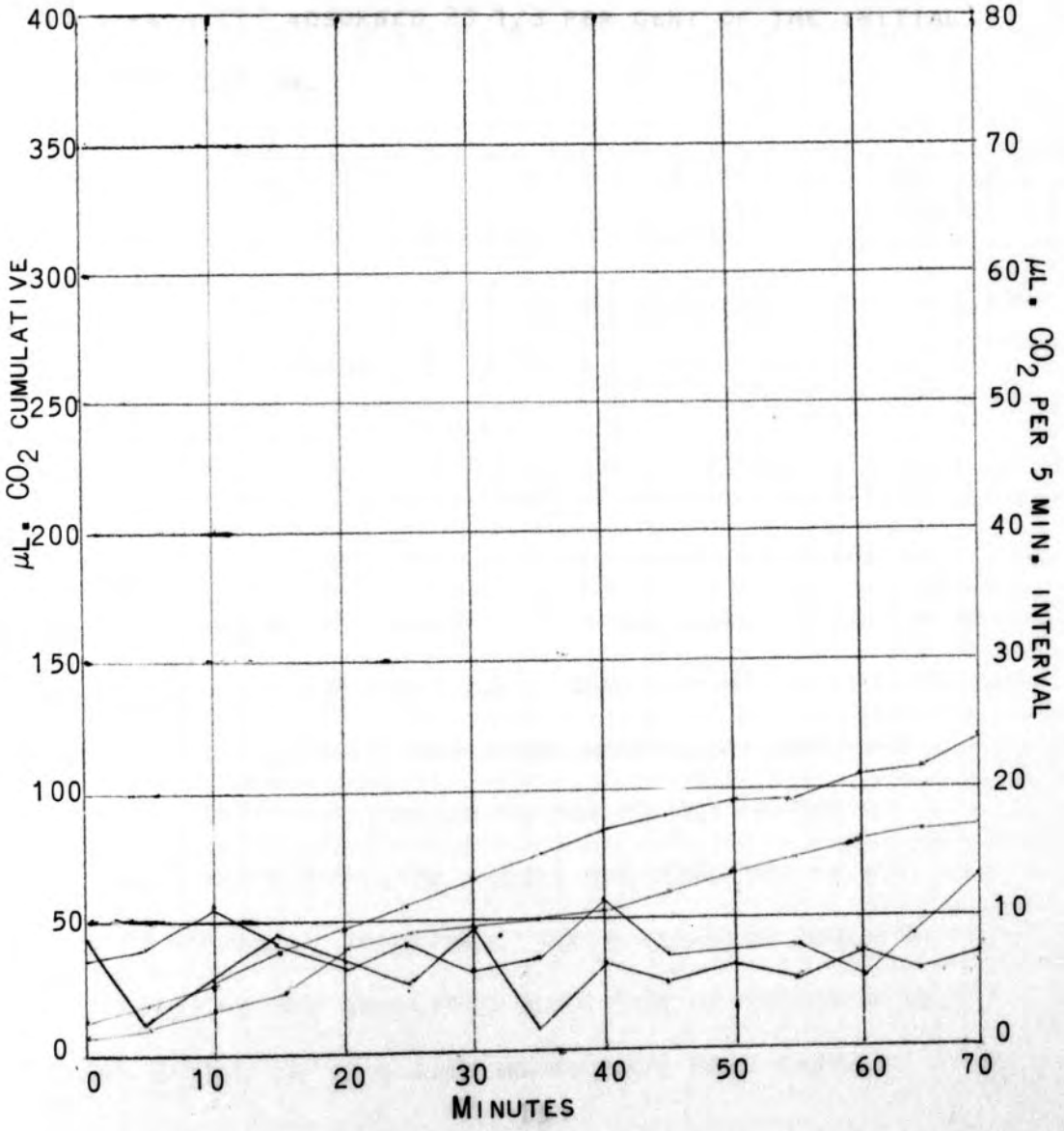


FIGURE III

PENICILLIN-PENICILLINASE ACTIVITY DETERMINED MANOMETRICALLY IN THE WARBURG CONSTANT VOLUME RESPIROMETER AT 37°C FOR STRAIN III STAPHYLOCOCCUS AUREUS

KEY: — μL. PER 5 MIN. INTERVAL WITH PENICILLIN
— μL. PER 5 MIN. INTERVAL WITHOUT PENICILLIN
— μL. CUMULATIVE WITH PENICILLIN
— μL. CUMULATIVE WITHOUT PENICILLIN



PLAQUE FORMATION WITH THE PHAGE PREPARATION OF THE INDIVIDUAL STRAINS OF BACTERIA; HOWEVER, WHEN THE PHAGE PREPARATIONS OF THE THREE STRAINS WERE MIXED TOGETHER, PLAQUES WERE OBSERVED WITH STRAIN II AND STRAIN III. IN TITRATION OF THE PHAGE PREPARATION, STRAIN II ADSORBED 39 PER CENT OF THE PHAGE POPULATION, AND STRAIN III ADSORBED 33 1/3 PER CENT OF THE INITIAL PHAGE POPULATION.

DISCUSSION

As stated above, the isolation and purification of staphylococcal penicillinase has not been perfected due to its intracellular nature. However, it is generally accepted that the production of penicillinase is the mechanism by which staphylococci become penicillin resistant. Partially purified penicillinase is prepared commercially from Bacillus cereus and other species which produce large quantities of the enzyme extracellularly. The reaction of partially purified penicillinase and penicillin gives the same gas evolution curve as was observed in this experiment.

The failure to find adsorption of phage on strain I could be attributed to the mutual exclusion effect seen in mixed infections since the phage preparation contained phages 80 and 81. In this case, if 80 and 81 are related phages, no plaque formation would be expected. Furthermore, when two non-lysogenic phages are used in mixed infection, the union may result in lysis. Moreover, these types of phages are reported to be highly lysogenic; therefore, their behavior could be attributed to the non-lytic condition of infectivity. Investigation in this area would have been carried

FURTHER IF THE PROPER MATERIAL HAD BEEN AVAILABLE AND

IF THE DATE OF REPORTING HAD NOT BEEN A LIMITING FACTOR.

CONCLUSION

From this experiment, the conclusion may be drawn that penicillinase production by penicillin-resistant *Staphylococcus aureus* may be related to the amount of phage adsorbed after a two-minutes adsorption period.

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