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Preparation and application of dry sensitivity discs to determine bacterial susceptibility to albamycin

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THE PREPARATION AND APPLICATION OF DRY SENSITIVITY DISCS TO DETERMINE BACTERIAL SUSCEPTIBILITY TO ALBAMYCIN

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TABLE OF CONTENTS

INTRODUCTION

A. Purpose of the Paper

Recently, the research laboratory of the Upjohn Company developed a new antibiotic, Albamycin,[1] produced by *Streptomyces niveus*, an actinomycete isolated from soil collected in Queens Village, New York (1,2). Dr. Gordon E. Gibbs, of the University of Nebraska Department of Pediatrics, was among the first to use this substance in clinical trials. The problem of devising a quick method for determining which patients had susceptible organisms immediately arose. The purpose of this paper is to report on a method for preparing and application of a satisfactory dry disc for the clinical investigation, together with available correlated tube dilution studies. Also, information which grew out of these studies concerning the *in vitro* spectrum of Albamycin is discussed. Finally, the clinical case reports completed at the time of preparation of this paper are presented.

B. History of Sensitivity Studies

The first bacterial sensitivity studies date back

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[1]Albamycin is the trade name of the Upjohn Company for the antibiotic *streptonivicin*. Albamycin and Cathomycin (Merck) are identical substances (3).

to the first antibiotic--namely, penicillin, first announced by Alexander Fleming in 1929 (4). He stated:

> "While working with staphylococcus variants a number of culture plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various microorganisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis.
>
> "Subcultures of this mould were made and experiments conducted with a view to ascertaining something of the properties of the bacteriolytic substance which had evidently been formed in the mould culture and which had diffused into the surrounding medium. It was found that the broth in which the mould had been grown at room temperature for one or two weeks had acquired marked inhibitory, bactericidal and bacteriolytic properties to many of the more common pathogenic bacteria."

Certainly the basis for future determinations of bacterial susceptibility to antimicrobiological agents was laid in the first efforts to determine the presence and potency of such agents in media seeded with organisms known to be susceptible. The first recorded method was Fleming's (4) which consisted of cutting a furrow in an agar plate and filling this with a mixture of equal parts of agar and broth in which the mould (penicillin) had grown. After this had solidified, cultures of various microbes were streaked at right angles from the furrow to the edge of the plate. After incubation he noted that the proximal portion of the culture for perhaps one centimeter became transparent and that in this portion

of the culture practically all of the microbes were lysed, showing that the antibacterial substance had diffused into the agar in sufficient concentration to bring about dissolution of the bacteria.

In this same article Fleming (4) stated that the inhibitory power of the agent could be accurately titrated by making several dilutions of penicillin in fresh nutrient broth and then implanting all the tubes with the same volume of bacterial suspension and incubating them. The inhibition could then be seen by noting the opacity of the broth. Similar methods are used in determining the potency of penicillin (5).

The next significant development was the Oxford Cup Method which was developed in 1941 (6). Small porcelain or glass tubes were set on the surface of solidified inoculated agar plates, filled with penicillin and incubated. Where the penicillin diffused out into the agar, growth of the test organism was inhibited and a circular clear zone resulted. The same principle was used in other bio-assay methods which are reviewed by Foster and Woodruff (7). A filter paper disc modification of the Oxford Cup Method was revealed by Vincent and Vincent in 1944 (8). Their principal changes lay in the substitution of a thick filter paper disc saturated with the penicillin solution for the small cylinder type of

receptacle used in the Oxford Cup Method. Bondi, Spaulding, et al. modified Vincent and Vincent's method in 1947 (9,10) by applying the wet disc directly to the agar plate inoculated with the fresh clinical specimen rather than first isolating the organism in pure culture as Vincent and Vincent had done.

Morley (11), who in 1945 was the first to report on the production and application of the dry sensitivity disc, used paper discs made from blotting paper and impregnated with penicillin before drying. He stated that this type of disc had the advantages of tolerating long-term storage and easy availability, as compared with the wet disc methods used by Foster and Woodruff and Vincent and Vincent. Subsequent articles discuss the credibility of the dry disc, its shortcomings, advantages, accuracy, and details of its preparation. Some of these will be taken up below in the discussion of the Albamycin disc effects. In reviewing the history of the development of methods for determining bacterial sensitivity to antibiotics one cannot fail to be impressed by how much of the groundwork was laid by Fleming in his first articles on penicillin (4,5). It would seem that much of the value of this phase of his contributions has been obscured by the potency of the agent he discovered, and further, perhaps, by a kind of reluctance to admit that

what was so carefully observed and reported in 1929 was so long in gaining proper recognition.

C. Product Information on Albamycin (1,2)

As indicated above, Albamycin is produced by an actinomycete isolated from a soil in Queens Village, New York. Albamycin is acid in character; it is active against both Gram-positive and Gram-negative bacterial infections in mice, including Staphylococcus aureus, Streptococcus hemolyticus, Diplococcus pneumoniae, Proteus vulgaris, Pasteurella multocida, Salmonella typhosa, and Klebsiella pneumoniae infections. Albamycin shows no cross-resistance either with clinical isolates or resistant organisms with any of the following antibiotics: penicillin, chlortetracycline, oxytetracycline, tetracycline, streptomycin, chloramphenicol, or erythromycin. Albamycin develops induced resistance in vitro at a slow, stepwise, penicillin-type rate rather than the rapid or streptomycin type of rate.

Albamycin is bactericidal in at least ten times the minimum inhibitory concentration. It is readily absorbed when administered orally. It is stable under acid, neutral and alkaline conditions and appears to be unaffected by passage through the stomach. It is excreted as a biologically active material in the urine.

For equal oral doses, per kilogram body weight, Albamycin gives blood levels in dogs approximately ten times higher than tetracycline, twenty-five times higher than erythromycin, and fifty times higher than penicillin. Absorption is such that peak concentrations are usually reached within two or three hours and appreciable levels persist beyond eight hours. These data have been confirmed by single-dose oral administration in humans. Safety for clinical trial of Albamycin has been established by toxicity studies in animals.

PREPARATION

A. Materials and Equipment

The following materials were used in the preparation of the dry sensitivity discs for Albamycin:

1. Filter paper discs (Fischer Scientific Co. 9-897, for the assay of penicillin and other antibiotics) ½ inch in diameter.
2. Automatic capillary pipette (Fischer Scientific Co., designed by Dr. Davis, Eli Lilly Co.) 13-696; .092 ml per delivery.
3. Albamycin Sodium (Streptonivicin Sodium) 250 mg. crystalline powder with 5.0 ml sterile diluent.
4. 200 ml of pH 7.8 0.1 M Phosphate buffer solution prepared from 17.0 ml of M/15 KH_2PO_4 and 183.0 ml of M/15 $Na2HPO_4$. pH was confirmed with a pH-meter after preparation of the solution (designated below as solution "x").
5. Path. specimen jars for storage.
6. 50 ml beakers; liter measuring flasks.

7. Pipettes, 1, 10, and 25 ml.

8. Calcium chloride desiccators.

9. 5.0 ml syringe with long 21-gauge needle.

10. 10 four-inch-square wire meshes with corners turned down to form legs to elevate the remainder of the mesh about ½ inch to be used as a platform for drying the discs in the desiccators.

All materials used in this procedure were first washed with distilled water, then autoclaved at 15 pounds pressure for twenty minutes.

B. Procedure

For this procedure reference is made to Tables I and II (pp. 35, 36).

Two ml of solution A (50 mg/ml) were added to 18.00 ml of X. The result was 20.00 ml of solution B (5 mg. or 5000 mcg./ml). Next, 2.00 ml of B were added to 18.00 ml of X, resulting in 20.00 ml of C (500 mcg./ml). Next, 8.00 ml of C were added to 8.00 ml of X, resulting in 16.00 ml of solution C-1 (250 mcg./ml). Then, 3.00 ml of C were mixed with 12.00 ml of X, giving 15.00 ml of solution C-2 (100 mcg./ml). Finally, 2.00 ml of C were added to 18.00 ml of X to give 20.00 ml of solution D (50 mcg./ml). This solution when delivered by the capillary pipette mentioned above gives a concentration of 4.6 mcg. per disc.

After the solutions were prepared, 30 to 35 discs were laid out on each of the wire meshes and solutions were delivered, one drop to each disc with the capillary pipette. Initially, 6 to 10 discs were made with each solution. After the discs had been impregnated, the wire meshes were placed in the desiccators and allowed to dry for 48 hours, after which time they were removed and placed in the storage bottles. Where possible, we kept the discs for storage in iceboxes at 40° F. As indicated below, however, this was not mandatory since the discs remain biologically active after room temperature storage up to 30 days.

The discs were prepared by this general method on three different occasions. The first run was on December 11, 1955. The solution (D) was then used for the second run on December 21, 1955. The final run was on January 23, 1956, at which time a modified procedure as outlined in Appendix II was used.

C. Bioassay of Discs

Before the preparation of this paper, but after all the discs had been made, it was decided to assay them for their diffusibility. The method is outlined in Appendix III. The results of the assay of 12 discs indicated that 3.12 mcg. diffuses from each disc in Brain Heart Infusion broth. This value is correlated in Table I (p. 35).

APPLICATION

The discs, prepared as outlined above, were distributed to the bacteriology laboratories of University and Children's Memorial Hospitals. These units supplied the material for the entries in Table III (p. 37) designated "field." Occasionally we used organisms which other laboratories, both in and out of Omaha, had found resistant. Those entries not marked "field" represent plates which were streaked from pure broth cultures, and most of these were prepared from field studies for subsequent tube dilution studies, although a few came to us from other laboratories already in pure culture, designated as resistant organisms, usually Staphylococci. All studies were done on blood agar. We attempted, but were not always successful in identifying the organisms we tested. On some occasions where microscopic examination was not helpful we were fortunate to have further studies done for a definite bacteriological diagnosis.

An effort was made to visit the "collecting" laboratories at least two or three times a week, but often this was not possible and it was necessary to take the verbal report of the technician on the 18-hour incubation result, or read the plate as long as 36 hours after seeding, or refer to the hospital chart. Those

plates streaked from broth culture were read personally by the author promptly at the end of 18 hours. In this manner the material of Table III (p. 37), which represents the body of the project, was obtained.

DISCUSSION

Before discussing the results of the use of the discs, three points about their preparation should be elaborated upon. The first is that the method of Morley (11) and Fairbrother, Martin, and Parker (12) was used with the essential modification being the substitution of wire meshes and calcium chloride desiccators for petri dishes and incubators for drying. We found that 48 hours in the desiccators at room temperature was ample for complete drying. No contamination resulted from the use of unsterilized desiccators. Finally, the use of desiccators freed the incubators for their intended function--an important consideration in a busy laboratory.

Secondly, the technique was quantitative throughout except for the use of the 5.0 ml syringe to transfer the standard diluent to the ampule containing the crystalline antibiotic and withdrawing the latter mixture to establish solutions A and B in Table I (p. 35). The error here would be that of failing to align the mark on

the syringe plunger properly on the calibration line of the barrel. Further, one might assert here that there would necessarily be a certain amount of dilution in using the same capillary pipette for delivering solutions of different concentrations. If there was error here, it was constant and calculated to be in the direction of greater dilution in order to obviate any illusion that the antibiotic or the disc was more potent than it really was. For this reason, when we were experimenting to find the best strength for the "base" disc, the solutions were delivered in ascending order of concentration.

Third and finally, one must consider a strictly human error that figured into the technique of preparation: the capillary pipette held only enough solution to cover about half of the discs on the wire mesh. Occasionally in refilling the pipette one would lose track where he left off in the line of identical white discs with the impregnation process. This error occurred less frequently if at all in later runs, but there still remains the possibility that three or four of each batch of discs were either unimpregnated or had received double exposure to the antibiotic solution. In general, however, with these few qualifications, after the procedure was adequately established, we found the method to be simple and convenient. (See Appendix II.)

The preparation of the disc posed the problems of selecting a suitable diluent for the crystalline antibiotic substance and then of determining an appropriate concentration to be used in the disc.

Our first choice for the diluent was sterile water since Upjohn (13,14) found that such a solution has a pH of 7.5 and remains stable at that pH. Furthermore, sterile water had been used satisfactorily in the tube dilution studies. Unfortunately, the discs prepared in this manner appeared to be biologically impotent. It cannot with any certainty be stated that the sterile water was the cause inasmuch as we were dealing with a number of hazards inherent in the first run of any procedure. Referring further to Upjohn product information (13,14), we recognized that Albamycin shows its greatest bioactivity at pH 7.8, and that in the agar diffusion assay studies Upjohn (15) had found a phosphate buffer solution pH 7.85 to be adequate. On the basis of this information we prepared a phosphate buffer solution of pH 7.8 (solution "X") which we found to be a satisfactory diluent throughout the project.

The next problem was to select the appropriate amount of Albamycin which, when in a dry disc placed on a freshly inoculated blood agar plate, would produce a readable area of inhibition among susceptible organisms.

We first wished to meet the standards of Eisenberg and Waggener (16) of using an antibiotic concentration on the disc which would parallel attainable blood levels. Upjohn (17) indicates that a dosage of 10 mg/kg given orally to humans resulted in serum levels ranging from 2.0 to 64.0 mcg./ml, depending upon the individual and the length of time after administration the serum sample was taken. With these values as a guide, we next set out to find what concentrations would give a readable zone of inhibition. It was inferred from further Upjohn information (15) that concentrations from 6.25 to 100 mcg./ml would be satisfactory for these had been used in the agar diffusion assay studies with good success. Accordingly, we mixed convenient dilutions of the clinical preparation (Table I, p. 35) and impregnated and dried discs with those solutions. Four test organisms, known to be susceptible, three Staphylococci and one Streptococcus, were streaked with a swab on blood agar plates and the areas of inhibition measured. These results are listed in Table II (p. 36). We found that the 4.6 mcg. disc, the smallest concentration used, produced an adequate zone of inhibition for our purposes. We were heartened to learn later that the Upjohn Company (18) had decided on a 5.0 mcg. disc, along with 30.0 and 100.0 mcg. for commercial preparation.

RESULTS

The purpose of our project was to produce a valid dry sensitivity disc for Albamycin. By valid disc is meant a disc which will consistently give results qualitatively as reliable as those of the tube dilution method. The disc was applied to 99 plates, about half of which were field sensitivity studies and the remainder studies on pure cultures. Forty of these 99 had concomitant serial dilution studies with Albamycin, and 75 per cent of these were done not only with Albamycin but also with the ten most common antibiotics to demonstrate the degree of resistance.

To evaluate the validity of the disc against the tube the value of 12.5 mcg./ml was arbitrarily selected as the greatest concentration in the serial dilution for minimal inhibition, for which we would designate the organism susceptible. This stipulation seemed to be compatible with the seemingly low concentration of antibiotic on the disc. In other words, where serial dilution showed inhibition at 25.0 mcg./ml, the disc was expected to show no inhibition.

Using the standards outlined above, we found the disc to be valid in 39 of 40 instances, or 97.5 per cent of cases in Table IV (p. 42) showed disc response in

agreement with the serial dilution result. In only one instance--Collar Staph, Table IV, C (p. 43)--was there disagreement, i.e., the disc indicated excellent susceptibility and the serial dilution by our standards indicated resistance. This disparity might well have been the product of the artificial standards we established, or the disc may have been one of those which had received double impregnation, or possibly factors of which we were not aware could have been present. It is felt, however, that Table IV (p. 42) is adequate evidence of the validity of the disc.

In the course of these studies we measured the diameters of inhibition wherever we used a disc. In the first instance we measured the zones (Table II, p. 36) to find out approximately what to expect in the routine studies (Table III, p. 37), and to be sure that the amount of antibiotic used was sufficient to produce a clearly visible zone. In Tables III (p. 37) and IV (p. 42) we measured the zones strictly as a matter of routine to make our observations as complete as possible. In order to simplify our report we instituted the following denotations for the size of the zones: N meant none, that is, no inhibition of growth; G meant good, and denoted an area from 13.0 mm (disc diameter 12.7) to 16.0 mm in diameter; and E for excellent denoted an area greater than 16.0 mm.

It should be emphasized that in recording these measurements we were fully aware of the hazards of attaching significance to them, keeping in full view the variables pointed out by Neter, Murdock, and Kunz (19) that marked differences in zones of inhibition occur with different strains and on different types of agar. We were aware that although we used blood agar throughout there still exist many differences among plates related to the specific circumstances under which they were poured or the agar mixed.

One notes, in examining Table IV (p. 42), the great disparity between zone diameters and corresponding serial dilution results. The same disparity is seen in perhaps greater relief in Table II (p. 36) where the discs with greater concentrations of antibiotics are recorded. Any number of paradoxical comparisons can be seen. Shattuck's and Brewer's Staphylococci gave identical endpoints in the serial dilution test, but the areas of inhibition for Brewer's organisms were larger at all strengths. Conversely, the penicillin-streptomycin resistant Staphylococcus aureus with a tube dilution endpoint of 1.56 consistently gave a larger zone of inhibition than the Streptococcus 8668 organism, the tube endpoint of which was 0.19. Any number combinations from Table II (p. 36) can be compared. These results bear out

well Lind and Swanton (20) who state that the alleged concentration of the disc is more important than a measured zone of inhibition, and Waggener (21) who states that all susceptibility tests are qualitative and serve only to differentiate between susceptible and resistant organisms. Also, although our conditions were not well controlled, these results tend to contradict Patrick, Craig, and Bachman (22) who state that a limited correlation exists between inhibition zones and the minimal inhibitory concentration as determined by serial dilution.

Our second conclusion is that the discs retain their bioactivity at least sixty days when kept under refrigeration. This evidence was derived from applying discs of the same vintage as those used when the material in Table II (p. 36) was developed and plates freshly seeded with Streptococcus 8668 sixty days later and measuring the zones of inhibition. They were almost identical in the original zones.

The remainder of our findings were incidental to the object of the project and concern the spectrum of Albamycin as we observe it. From Table III (p. 37) it will be observed that Albamycin had no effect in vitro against the six coliform organisms, no effect against the single Proteus vulgaris, no effect with four Pseudomonas, and no effect against the five Streptococcus fecalis. Ten of

fourteen Streptococci (excluding fecalis) were resistant. One diphtheroid was susceptible and another was not. The Staphylococci were emphatically susceptible. Table V (p. 44) shows the results with 30 Staphylococci which demonstrate strong resistance to one or more of the common antibiotics. These results agree essentially with the Upjohn Company results using Brain Heart Medium (23).

CLINICAL SUMMARIES

Presented below are the clinical summaries on twelve pediatric patients[1] who received Albamycin. The dosage schedule was 50 mg/kg/day. The Albamycin was administered orally in 100 and 250 mg. capsules. Because of the elevated blood NPN determinations encountered initially with Osborn (Case No. 1) and Brewer (Case No. 3), all NPN's available at the time of preparation of the summaries are recorded. At the end of each summary is a short comment on the observed effect of the Albamycin along with appropriate remarks about the sensitivity studies, where those are available. Finally, the substance of these comments is consolidated into tabular form at the end of the histories. It should be emphasized

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[1]Eleven of the patients were from the University of Nebraska College of Medicine Department of Pediatrics (both Dispensary and Hospital) and one was seen at Children's Memorial Hospital.

that the purpose of this paper was to prepare a valid disc, but it seemed fitting to present those clinical cases available and arrive at some rough conclusion about the *in vivo* efficacy of Albamycin therapy.

CASE REPORTS

Case No. 1 (Joyce Osborn, 1787)

This 13-month-old white female entered University of Nebraska Hospital on 9-26-55 for the second time with the history of a respiratory infection for two months, and a pneumonitis and fever. Physical examination showed her temperature to be 101°, pulse 150, and respirations 44. A few coarse rhonchi were heard throughout the chest and high-pitched rales were heard on the lower aspect of both lungs, more marked on the right. Her WBC was 31,300. A tentative diagnosis of septicemia, mental retardation and hypothyroidism was made. Among other therapeutic measures taken, penicillin, streptomycin, and chloramphenicol and achromycin were given at various times, but discontinued until 10-11-55, when Albamycin 100 mg. q 4 hrs. was started, because her temperature spiked to 103.2°. Her WBC on 10-10 was 12,000. Her temperature on 10-11 was 101.5°, but after 10-12 levelled to a range of 98.0° to 99.6°, where it remained until 10-24. Her WBC 9,000 on 10-14; 15,100 on 10-15. Albamycin was discontinued on 10-25. Throughout her hospitalization numerous blood, urine, and throat cultures were done. No disc studies with Albamycin were done except on a pure culture of throat Non-hemolytic Micrococcus pyogenes var. aureus on which tube dilution studies were done on 10-11-55, and showed the organism to be inhibited at 0.19 mcg./ml.

Shortly after discontinuing the Albamycin the fever resumed. This was first treated with Gantrisin, then achromycin, and then streptomycin and penicillin. 8.0 ml of immune serum globulin was given in an effort to raise her antibody level. On 11-25-55 all medication was discontinued and she was placed again on Albamycin, 100 mg. q 4 hrs. The fever spiked to 103° until 12-4 when it levelled out to normal. The WBC was 15,600 on 10-25; 14,550 on 11-10; 20,000 on 11-21; 14,000 on

11-28; and 8,600 on 12-6. Albamycin was discontinued on 12-9-55 after the patient had been afebrile for four days. She was discharged on that date with the diagnosis above after 74 days of hospitalization.

Blood NPN Levels:	10- 1:	60 mg %
	10-10:	38.5
	10-14:	28.5
	10-15:	40.2
	10-20:	46.8
	11-28:	50.5

Comment

This is a unique case for showing the results of temporarily withholding Albamycin where other antibiotics seem ineffective. During the first course of therapy the temperature drop was immediate and fairly constant. The WBC dropped to normal range but after four days was higher than before the agent was given. During the course between 11-25 and 12-9-55 the response was similar--the fever yielded but the WBC persisted until the last week of therapy when the patient's own defenses may have been adequately mobilized. Though possibly valid in this case, the sensitivity studies for Albamycin were not clinically helpful. The clinical response was very good.

Case No. 2 (Robert Lipton, 8661)

This 11-year-old white male had been seen at the University of Nebraska Dispensary every two months since 1-4-55 with a diagnosis of cystic fibrosis of the pancreas. His weight since 9-27-55 had stayed around 44 pounds. He had been treated at the Dispensary from

time to time for respiratory infections with terramycin, neomycin, bacitracin, and achromycin. He was seen on 11-26-55 with the chief complaints of much coughing in the morning and generally not feeling good. Physical examination showed cyanosis with respiratory distress, respirations 40, pulse 140, and a temperature of 101°. His WBC was 38,700. No bacteriological studies were done. He was placed on Albamycin, 250 mg. q 6 hrs. He returned on 12-2-55 subjectively feeling much better, but his WBC was 37,200. He was then placed on neomycin and his chest cleared up and his WBC came down to 10,500 by 12-10-55. He was seen next on 1-24-55. He had used up the neomycin a week previously. His weight was 48 pounds, his chest was almost clear except for a few faint crackles in the lower left. His WBC was 16,900. Bacteriological studies were done on 1-24-55 and revealed a Micrococcus pyogenes var. aureus, a non-hemolytic Streptococcus, and a Neisseria. Disc studies showed "partial" inhibition with Albamycin on the field plate, but none with the Micrococcus. On 1-28-56 he was restarted with Albamycin, 250 mg. q 6 hrs. His WBC at this time was 24,000. A WBC done on 1-31 was 13,100. He was supposed to come in on 2-4-56 for another check but he refused because the visit would interfere with his playing.

Blood NPN on 12-16-55 : 33.2 mg %.

Comment

In the first instance one week of Albamycin therapy did not alter a rather high WBC but did make the patient feel better. In the second instance, with laboratory studies being equivocal, if not negative, Albamycin almost halved the WBC and made the patient feel well enough not to want to come in.

Case No. 3 (Betty Ann Brewer, 7428)

This 4-year-old colored female entered University of Nebraska Hospital on 11-28-55 with the chief complaints of vomiting and lethargy beginning 2½ hours before admission and a cold for one week. Physical

examination showed the left tympanic membrane to be injected, the tonsils large and inflamed. There was marked nuchal rigidity--flexion of the neck caused flexion of the lower extremities. It was not possible to elicit the knee jerks; the Kernig was positive but the Babinski was absent. The WBC was 14,100 with 50 staffs; temperature was 103.4°; the urine was negative except for 4/ acetone. A presumptive diagnosis of meningitis was made and a spinal tap done. Patient was placed on penicillin, chloramphenicol and streptomycin. The spinal tap culture revealed Hemophilus influenza organisms. On 11-29 the WBC was 27,900; on 11-30, 18,900. By 12-14, the 16th day, all previously ordered antibiotics except for chloramphenicol were discontinued and Albamycin 100 mg. orally q 4 hrs. was started, to combat a spiking temperature which had begun on 12-9 (11th day) and had ranged from 103.2° to 100°. The WBC at the time the Albamycin was started was 6,000 and the temperature 101°. On the same day the temperature returned to normal. In spite of the administration of Albamycin and the continuance of chloramphenicol, while the temperature remained normal after 12-14, the WBC sent to 7,800 on 12-15, and to 12,000 on 12-19. Tube dilution studies revealed that Albamycin was effective at the 12.5 mcg./ml level against throat organisms and 0.19 against the urine organism, taken 12-13. Child was discharged on 12-29-55.

Blood NPN Levels: 11-29: 60 mg %
12-14: 39

Comment

This represents a case wherein Albamycin seemingly brought the temperature to normal but the WBC became elevated. Evaluation is not clearcut because of concomitant administration of chloramphenicol. Sensitivity studies were of little value on an a priori basis, although throat culture revealed Micrococcus pyogenes aureus 12-13. This appears to have been an

instance wherein the primary infection was due to Hemophilus influenza and a Micrococcus infection was superimposed.

Case No. 4 (Donald Shattuck, Children's Memorial Hospital)

This 10-year-old white male has been followed at the Hattie B. Munroe Home for Convalescent Children by Dr. Gibbs. In 11-48 he was diagnosed fibrocystic disease of the pancreas and has been hospitalized at the Children's Memorial Hospital ten times since, usually for respiratory complaints. He is currently underdeveloped and undernourished, weight 42 pounds. His last CMH admission ended on 11-15-55. On 12-12-55 physical examination revealed increased crackles on both sides of his chest, a weak cough and some dyspnea. His WBC was 16,000. Albamycin, 250 mg. q 6 hrs., was given for one week. On 12-19 his chest was the same, his WBC was 16,000, and his weight 43 pounds. On 1-22-56 his WBC was 14,500 and increased pulling in his respirations was noted. On 1-30-56 his WBC was again 16,000, moist crackles were heard in the chest. Albamycin, 250 mg. q 6 hrs. for one week, was again started.

Comment

Except for a one-pound weight gain and questionable clinical improvement, there was no improvement in this patient while he was on Albamycin therapy.

Case No. 5 (Barbara Moylan, Children's Memorial Hospital)

This 19-month-old white female entered Children's Memorial Hospital on 12-26-55 with a 7-month history of diarrhea, cough, and poor weight gain. X-rays showed bilateral maxillary sinusitis with thickened membranes, bilateral basal bronchitis and peribronchitis, with bronchopneumonia in the left lower lobe over the diaphragm. Her admission WBC was 17,900, temperature was 99.0°. Nose and throat cultures revealed Micrococcus pyogenes var. aureus which was inhibited by the disc. Tube dilutions showed inhibition at 0.19 mcg./ml. On 12-28 she was

started on Albamycin 100 mg. q 6 hrs. Her chest cleared appreciably. She was discharged on 12-30-55, with a WBC of 18,000, and took Albamycin with her. She was seen on 1-4-56, with a WBC of 9,000.

Comment

This case shows a good clinical response and WBC decrease with Albamycin. The sensitivity studies correlated well with the response.

Case No. 6 (Harry Carr, 7853)

This 7-month-old negro male entered University of Nebraska Hospital on 12-30-55 with a runny nose and high fever. (No history was available.) On physical examination he appeared to be listless and quite ill with a red, scaly papular rash over the scalp, neck, ears, and forearms. His right ear drum was extremely red with no light reflex. His left ear drum was red-ringed but the light reflex was intact. His throat showed 2/ injection of the pharynx and tonsils. His temperature was 102.2° and his WBC was 11,300. A diagnosis of acute otitis media, sinusitis, and pharyngitis with infantile eczema was made. Albamycin, 100 mg. q 6 hrs., was started. Throat culture revealed Micrococcus pyogenes var. aureus and a Neisseria species on field studies with "partial" inhibition with the disc (complete with penicillin). Tube studies revealed inhibition with 0.39 mcg./ml Albamycin. The patient's temperature dropped to 99.0° within 12 hours after the Albamycin was started, spiked to 101° within 24 hours, then slowly levelled to near normal thereafter. On 1-4-56 he had a WBC of 4,500. He was discharged on 1-6-56, much improved.

Blood NPN: 1-4-56: 37 mg %.

Comment

This case illustrates a good clinical response, temperature response and WBC decrease with Albamycin. The disc here gave a misleading impression on field

studies, but the tube studies and disc results with the pure culture were compatible with the clinical result.

Case No. 7 (Sharon Mason, 5626)

This 6-year-old white female had been seen at the University of Nebraska Dispensary for cystic fibrosis of the pancreas every two to four weeks throughout 1955. She came in on 1-3-56 complaining of a cough, runny nose, and poor appetite. A 3-pound weight loss was noted. On physical examination she seemed to be slightly breathless. There was cyanosis of the lips and clubbing of the fingers. There were a few rales in her chest over the right base. A chest film showed bilateral bronchitis with interstitial pneumonitis. Her WBC was 17,100. She was placed on Albamycin, 100 mg. q 4 hrs. Sputum culture revealed Hemolytic micrococcus aureus[1] with complete inhibition of growth on the plate by the Albamycin disc. On 1-9-56 her appetite had improved and a 2-pound weight gain was noted. Her chest showed only a few crackles and her WBC was 13,700. Since then she has been on oral penicillin. Sensitivity studies on 1-24 showed "partial" inhibition on the "field" plate with the disc, a "trace" of inhibition with the B-hemolytic Micrococcus aureus and "complete" inhibition with the B-hemolytic Micrococcus citreus.

Blood NPN: 1-11-56: 35 mg %

Comment

A six-day course of Albamycin therapy in this case resulted in a good clinical response and a noticeable decrease in the WBC. The sensitivity studies were compatible with the clinical result obtained.

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[1]Tube dilution studies down from a throat culture taken on 10-11-55 revealed no growth at the greatest dilution--0.19 mcg./cc. The organism was hemolytic Micrococcus pyogenes var. aureus.

Case No. 8 (Timothy Willet, 5959)

This 6½-year-old white male entered University of Nebraska Hospital on 12-30-55 apparently free of infection for cardiac catherization. He developed an acute pharyngitis on 1-5-56, after his temperature had spiked to 101.7° the day before. His WBC was 9,000 on 1-5. Albamycin, 250 mg. q 6 hrs., was started. On 1-6 his WBC was 10,300; on 1-9, 8,000; and on 1-14, 10,000. On 1-10-56 a note was made that his clinical response to Albamycin had been good. (Bicillin was started on 1-11, the day of the catherization.) No throat cultures were done.

Comment

This case responded well clinically to Albamycin. The slight drop in his WBC may or may not be significant. No culture and sensitivity studies were done.

Case No. 9 (Dot Chatman, 4861)

This 7-month-old white female entered University of Nebraska Hospital on 1-5-56 for the second time with the chief complaints of cough, fever, and runny nose for the past day. Physical examination showed the right ear and canal to be inflamed, a large amount of mucous discharge from the nose, and the tonsils to be inflamed. Her admission temperature was 100.8°. WBC was 14,600. With a diagnosis of interstitial pneumonitis, bilateral bronchitis, and pharyngitis with a secondary otitis media she was placed on Albamycin 100 mgm q 6 hrs. WBC on 1-6 was 7,000; on 1-9, 7,700. By 1-10 her temperature was down to 99.8°. She was dismissed on 1-11-56 with a normal temperature and negative physical findings. No sensitivity studies were done. Dismissed on 1-11-56 with a normal temperature.

Comment

This child showed a good clinical response to Albamycin. Her temperature, WBC, and physical findings improved considerably.

Case No. 10 (Betty Taylor, 7719)

This 3-month-old colored female entered University of Nebraska Hospital on 12-21-55 with the chief complaint of having "turned blue" for a period of 45 minutes about three hours before admission. She had had a cold for two days before admission. Physical examination showed her respirations to be "grunty and raspy" in character, her nose and throat full of mucus and "squeaks" in the right chest. Her temperature rectally was 99.0°; WBC was 12,000; her urine was negative. A diagnosis of bronchitis was made and therapy consisting of Bicillin, achromycin, and steam tent was started. Her temperature line stayed flat, but by 1-10-56 she still had rales in her chest and her WBC was 12,700. On 1-8-56 the achromycin had been stopped and Albamycin, 50 mgm. q 4 hrs., was given for two days, in spite of the fact that the laboratory sensitivity reports[1] were equivocal. Although the WBC on 1-13-56, her day of dismissal, was 13,400, the patient's rales in the chest, stuffy nose, and respiratory infection clinically improved markedly.

Blood NPN: 1-10-56: 44 mg %.

Comment

This is a respiratory infection which failed to yield to Achromycin-Penicillin therapy, but clinically improved with the substitution of Albamycin for Achromycin. There was an equivocal report from the laboratory as to sensitivity. The WBC and temperature were not appreciably affected.

Case No. 11 (Cynthia Andrews, 1617)

This 6-month-old white female entered University of Nebraska Hospital on 12-30-55 with the chief complaint of a "rash" and being "fussy" for the past day, having

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[1]Laboratory studies showed organism from throat culture taken on 1-10-56 to be non-hemolytic streptococcus and non-hemolytic Staphylococcus. No inhibition by Albamycin disc; tube dilution 12.5.

suffered intermittently during the past two weeks with a "cold." On physical examination she was found to have some respiratory distress, a "general blotchy appearance," ptosis of the left eyelid, but a normal neurological examination. Her rectal temperature was 102.8°; her urine, negative; but her WBC was 32,800. It was felt that she had a septicemia, probably a meningococcemia, and she was placed on a regimen of gantrisin, penicillin, cortisone, and supportive measures as indicated. Her temperature came to normal after 12 hours, but spiked to 102.9° on the 5th day. Her WBC, taken on the 6th day, was 27,800. On the 7th day the laboratory reported Micrococcus pyogenes var. aureus in the throat and blood cultures, and Neiserria meningitides also in the blood culture. The temperature spiked to 101.2°.

On the 9th day (1-8-56) the child's temperature was 101.6° in the evening. ENT and chest were negative. The fever was thought possibly to be due to absorption of necrotic material. On the basis of the finding of Staphs on the throat culture--but no growth inhibition by the disc--all other antibiotics were stopped and Albamycin, 100 mgm. q 4 hrs., was started. On 1-10-56 her WBC was 23,900; on 1-13-56, it was 10,600. Albamycin was discontinued after 8 days. After minor spikes within 12 hours of the beginning of Albamycin therapy, her temperature levelled to normal where it remained until she was discharged on the 20th day. Her last WBC was on 1-6-56: 11,900. No more Albamycin was given. She was discharged 1-19-56.

Blood NPN: 1-16-56: 41.0 mg %.

Comment

This is a case of meningococcemia complicated with a Staph infection in the throat. Albamycin here brought the WBC from 23,000 to 10,000, and brought the temperature to normal. Correlation with the dry disc was unsatisfactory. (Throat culture on 1-9-56 revealed no inhibition with Albamycin disc.)

Case No. 12 (Le Ann Durtin, 7759)

This 4-month-old white female with a diagnosis of fibrocystic disease of the pancreas was readmitted to University of Nebraska Hospital on 1-9-56 with respiratory symptoms and vomiting and diarrhea. Her chest showed inspiratory wheezes but no dullness or rales. Her temperature was 103.5°, but went down to 100° after 8 hours. Her WBC was 21,200. Throat cultures were taken and showed on field plates to be predominantly coliform and some Micrococcus pyogenes. No disc or tube studies were done. She was started on Albamycin, 50 mg. q 4 hrs., on 1-9-56, which was continued 2 days until laboratory studies were returned. Her WBC on 1-10-56 was 11,200. After 2 days her temperature levelled out to the 98-99.5° range. Albamycin was discontinued because of predominance of Coliforms on the field plate. She has not received Albamycin since but she is still in the hospital.

Blood NPN: 1-11-56: 64.5 mg %.

Comment

This child showed a reasonable WBC response, but there is a possibility that this response may have been accomplished without the antibiotic.

CHART A

SUMMARY OF SALIENT POINTS CONCERNING THE CLINICAL RESULTS OF THE USE OF ALBAMYCIN IN THE CASES CITED ABOVE

Patient Case No.	WBC Decr.	Temp. Decr.	Clinical Response (Subjective effects)	Correlation with LAB	General Estimate
1	None until late	Good	Very good	No disc, but good tube cor.	Very good#
2	A. None B. Good	No record No record	Good Good	No studies. Poor disc result	Fair Good
3	None	Good	No record	Not clear-cut because of simultaneous chloramphenicol therapy	Not clear-cut--poss. good
4	None	None	Questionable	No studies with Albamycin	Probably not effective
5	Good	Good	Good	Ideal	Good#
6	Good	Good	Good	Tube cor. good, but disc not satisfactory	Good#
7	Good	Good	Good	Good	Good#
8	Slight	No adequate comparison	Good	None	Good#

#By the rough standards used these cases are considered to have obtained definite benefit from Albamycin treatment.

CHART A--continued

Patient Case No.	WBC Decr.	Temp. Decr.	Clinical Response (Subjective effects)	Correlation with LAB	General Estimate
9	Good	Good	Very good	None	Good#
10	Not clear-cut	Not clear-cut	Not clearcut	Lab reported "partial" inhibition by disc	Questionable (Simultaneous penicillin adm.)
11	Good	Good	Good	None	Good#
12	Good	Fair	Questionable	Poor	Questionable

CONCLUSIONS

1. A simple valid method of preparation of a dry sensitivity disc for Albamycin is presented. On the basis of the material presented, the disc was 97.5 per cent efficient by comparisons with serial dilution studies.

2. Under refrigeration the disc retains its bio-activity for at least sixty days.

3. *In vitro* Albamycin had no effect against the coliform organisms, *Streptococci fecalis* and *Pseudomonas* organisms tested.

4. Albamycin is effective *in vitro* against many resistant *Staphylococci*.

5. Of twelve clinical cases presented there seemed to be evidence that seven definitely benefited from treatment with Albamycin. No evidence of drug toxicity, especially with regard to kidney function as indicated by blood NPN determinations, was observed.

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To Miss Leni Rauschenberg, Medical Technologist, University of Nebraska College of Medicine

To Miss Mary Jane Herbert, Chief Laboratory Technician, Children's Memorial Hospital

APPENDIX I

TABLES

TABLE I

ANTIBIOTIC SOLUTIONS ARE SHOWN BELOW WITH THE VARIOUS CORRESPONDING CONCENTRATIONS TO AID READER IN FOLLOWING THE METHOD

Solution	Concentration mg or mcg/ml	Relative Strength Using D as Unity	Microgram/disc Applied	Bioassay mcg/disc
A	50 mg/ml 50,000 mcg/ml	1000 s	4,600 mcg	3120
B	5000 mcg/ml	100 s	460 mcg	312
C	500 mcg/ml	10 s	46 mcg	31.2
C_1	250 mcg/ml	5 s	23.0 mcg	15.6
C_2	100 mcg/ml	2 s	9.2 mcg	6.25
D	50 mcg/ml	1 s (unity)	4.6 mcg	3.12

TABLE II

ZONES OF INHIBITION OBTAINED WITH VARIOUS STRENGTH DISC, USING SUSCEPTIBLE ORGANISMS. THIS WAS DONE TO DETERMINE WHAT CONCENTRATION OF ANTIBIOTIC MIGHT BE NEEDED TO MAKE A DISC POTENT.

		Zone of inhibition in mm around discs of different strength as indicated					
Patient and/or Organism	Tube dilution	1 s	2 s	5 s	10 s	100 s	1000 s
Shattuck Staph	0.19 mcg	3.0	4.0	4.0	6.5	8.0	9.0
Brewer Staph aureus	0.19	3.5	4.5	5.0	7.0	9.0	12.0
Strep 8668	0.19	1.0	3.0	3.0	4.0	6.0	8.0
Staph aureus Resistant to Penicillin & Strep	1.56	4.0	4.0	4.0	6.0	9.0	9.0

SEE TABLE I for specific amount of antibiotic on each disc with its diffusibility.

TABLE III

ALBAMYCIN DRY DISC SENSITIVITY RESULTS

Patient	Organism	Material Streaked from	Disc Effect	Tube
				mcg/ml
Shattuck	Staph	Broth	G	0.19
Brewer	Staph aureus	Broth, urine	G	3.12
Test org.	Strep 8668	Broth	E	0.19
Unknown	Resistant staph	Broth	E	1.56
Allison	Strep fecalis	Broth	N	R to 100
Brewer	Staph	Broth, throat	G	12.5
Lee	Staph	Broth	E	0.78
BP	Staph	Broth	E	6.25
AJ	Staph	Broth	E	1.56
Collar	Staph	Broth	E	25
Haveat	Staph	Broth, cellulitis	E	1.56
Carr	Strep	Broth	E	0.39
Murphy	Staph II	Broth	G	1.56
Trust	Staph	Broth	E	0.78
Murphy	Staph I	Broth	G	1.56
Cummings	Staph	Broth	G	0.39
OZ	Staph	Broth	E	1.56
Winkler	Strep	Broth	N	R to 50
Shipman	Staph	Broth	E	6.25
Lipton	Staph	Broth	G	.095

TABLE III--continued

Patient	Organism	Material Streaked from	Disc Effect	Tube
Moylan	Staph aureus	Broth, nose and throat	G	0.19
Mason	Hem.staph aureus	Broth, sputum	E	0.19
Carr	Staph aureus	Broth, throat	G	0.39
Farris	?	Field, nose	G	--
		" throat	E	--
	Coliform	Arm abscess	N	--
	Staph	" "	N	--
Dutscher	Anerobe	Field, urine	N	--
Lovejoy	B-hem.strep	Field, urine	E	--
Wray	Staph	Field, ?	E	--
Webb	?	Field, throat	N	--
Benianato	Yeast	Urine	N	--
Cummings	Strep	Urine	N	--
Cain	Staph	Broth,arm stump	G	25.0
Farris	B-strep	Broth	N	--
Cain	B-strep	Broth	N	--
Farris	Staph	Broth, throat	G	--
6966	Staph albus	Broth,pilonidal	G	--
A153	Staph	Broth	E	--
6990	?	Field, urine	G	--
6994	Strep fecalis	Broth, urine	N	R to 100
6987	Staph aureus	Broth, gluteal abs.	E	0.78

TABLE III--continued

Patient	Organism	Material Streaked from	Disc Effect	Tube
6977	Strep fecalis	Broth, urine	N	R to 100
Andrews	Gram neg. rod	Field, NP	G	--
Price	Strep	Field, BM	N	--
Garner	Staph albus	Field, empyema	G	--
	B-hem. Staph	Field, urine	G	--
	B-hem. Strep	Field, urine	N	--
	Pseudomonas	Field, empyema	N	--
Garcia	Staph	Field, rt. ear	G	--
	Pseudomonas	Field, "	N	--
	Gram neg. rod	Field, "	N	--
Hoffman	Alpha hem. strep	Blood culture	N	--
Pittock	Coliform	Field, NT	N	--
Christensen	Coliform	Field, NT	N	--
Schwank	Hem. staph	Field, NT	N	--
Christensen	Pseudomonas	Field, throat	N	--
Burns	Coliform	Field, throat	N	--
Christensen	Staph	Field, nose	NE	--
TAYLOR	Staph	Broth, throat	G	12.5
	Strep	" "	G	12.5
Burda	Staph aureus	Field, pus	G	--
	Staph albus	" "	G	--
	Pseudomonas	" "	N	--
Cole	E. Coli Communior	Field, urine	N	--
Klapka	?	Field, urine	N	--
Richardson	Staph	Field	G	.039

TABLE III--continued

Patient	Organism	Material Streaked from	Disc Effect	Tube
Miller #40	Staph	Field	E	.039
Channel	Staph	Field, urine	E	1.56
Taylor	?	Field, throat	N	--
Easter	Staph	Field, cervix	G	--
R. Jones	?	Field, throat	N	--
Parson	Diphtheroid	Field, ulcer	G	--
	Staph	" "	G	--
Brummeier	?	Field, wound	N	--
Garcia	Gram - rod and + cocci	Field, lt. ear	N	--
	Proteus	" " "	N	--
Vandusen	Diphtheroid	Field, urine	N	--
Trip	Gram - rod	Field, urine	N	--
Taylor	Strep	Field, urine	N	--
Smith	Strep	Field, urine	N	--
Loften	Staph	Field	E	--
Mead	Staph	Field, furuncle	E	--
Burman	Hem. Staph	Field, urine	G	--
Bott	Non-hem. staph	Field	G	--
Sorenson	Strep viridan	Field	N	--
	Coliform	Field	N	--
Pitzel	Staph	Broth	G	3.12
Harris	Strep fecalis	Broth	N	--
Harris	Staph aureus	Broth	G	--

TABLE III--continued

Patient	Organism	Material Streaked from	Disc Effect	Tube
7031	Staph	Broth	E	0.19
7035	Staph	Broth	E	0.78
7025	Staph	Broth	E	1.56
7013	Staph	Broth	E	0.39
7020	Staph	Broth	E	0.78
#5	Pfizer staph	Broth	E	0.19
Unknown	B-strep	Broth	N	R to 100
Cain II	Strep fecalis	Broth	N	25.0

TABLE IV

DISC EFFECTS WITH CORRESPONDING TUBE DILUTION RESULTS
40 STUDIES

A. No Effect with Disc, 6 Studies

Cain II	Strep Fecalis	R to 25
Winkler	Strep	R to 50
Allison	Strep Fecalis	R to 100
6994	Strep Fecalis	R to 100
6977	Strep Fecalis	R to 100
Cain	Strep Fecalis	R to 100

B. Good Effect with Discs, 15 Studies

Shattuck	Staph	0.19
Lipton	Staph	0.19
Mason	Staph	0.19
Moylan	Staph	0.19
Brewer (urine)	Staph	0.19
Cain	Staph	0.19
Cummings	Staph	0.39
Carr	Staph	0.39
Richardson	Staph	0.39
Murphy	Staph I	1.56
Pitzel	Staph	3.12
Brewer (throat)	Staph	12.5
Taylor	Staph	12.5
Taylor	Strep	12.5

TABLE IV--continued

C. Excellent Effect with Discs, 21 Studies		
#5	Staph	0.19
7031	Staph	0.19
Test Organism	Strep 8668	0.19
Carr	Strep	0.39
Miller #40	Hem. Staph Aureus	0.39
7013	Staph	0.39
Lee	Staph	0.78
Trust	Staph	0.78
6987	Staph	0.78
7020	Staph	0.78
7035	Staph	0.78
Unknown	Res. Staph	1.56
OZ	Staph	1.56
Channel	Staph	1.56
7025	Staph	0.19
Haveat	Staph	1.56
AJ	Staph	1.56
Shipman	Staph	6.25
BP	Staph	6.25
Collar	Staph	25.0

TABLE V

A COMPOSITE OF THOSE STAPHYLOCOCCI FROM TABLE IV, B AND C WHICH WERE FOUND BY TUBE DILUTION STUDIES TO BE RESISTANT TO OTHER ANTIBIOTICS BUT SUSCEPTIBLE TO ALBAMYCIN #

Name	Alba-mycin	Erythro-mycin	Neo-mycin	Peni-cillin
Shattuck	0.19	3.12	0.19	25.0
Lipton	0.19	0.19	25.0	100
Mason	0.19	6.25	0.78	25.0
Moylan	0.19	0.19	100	1.56
Cummings	0.39	0.78	0.19	0.78
Carr	0.39	0.19	3.12	100
Richardson	0.39	0.78	0.78	100
Brewer I	0.19	0.19	0.19	100
Murphy	1.56	50.0	3.12	100
Pitzel	3.12	0.39	0.39	100##
Brewer II	12.5	100##	3.12	100##
Taylor	12.5	1.56	6.25	50.0
Cain	0.19	0.19	0.19	100##
7031	0.19	0.78	6.25	100##
Miller #40	0.39	0.19	6.25	50.0
7013	0.39	0.39	0.19	100##

Those values which are 50 mcg./ml or greater in the tube studies are underlined.

Resistant to 100 mcg./ml.

TABLE V--continued

Bacit-racin	Strepto-mycin	Chloram-phenicol	Aureo-mycin	Terra-mycin	Tetra-cycline
1.56	100##	12.5	100	100	100
6.25	100	25.0	--	100	50
3.12	100##	12.0	50.0	100	100
6.25	50.0	6.25	6.25	12.5	25.0
0.39	3.12	1.56	6.25	3.12	3.12
3.12	100##	12.0	50.0	100	100
6.25	100	6.25	100	100	100
3.12	100	100	50.0	100	100
12.5	100	50.0	100##	100##	100##
3.12	100##	6.25	50.0	100##	100##
3.12	100##	50.0	100##	100##	50.0
3.12	100##	100##	100##	100##	100##
3.12	100##	12.5	100	100##	50.0
1.56	100##	3.12	50.0	100	100
6.25	100##	6.25	50.0	50.0	25.0
3.12	100##	3.12	25.0	50.0	25.0

TABLE V--continued

Name	Alba-mycin	Erythro-mycin	Neo-mycin	Peni-cillin
Lee	0.78	100	0.19	3.12
Trust	0.78	100##	1.56	100##
6987	0.78	1.56	6.25	100##
7020	0.78	12.5	3.12	100##
7035	0.78	0.78	6.25	100##
Res. Staph	1.56	0.19	0.19	100##
OZ	1.56	100##	25.0	100##
Channel	1.56	3.12	0.39	100##
7025	0.19	1.56	3.12	100##
Haveat	1.56	0.19	0.78	100##
AJ	1.56	100##	12.5	100##
Shipman	6.25	100##	0.19	100##
BP	6.25	50.0	0.19	100##
Collar	25.0	1.56	25.0	6.25

TABLE V--continued

Bacit-racin	Strepto-mycin	Chloram-phenicol	Aureo-mycin	Terra-mycin	Tetra-cycline
0.19	100##	3.12	25.0	100	100
1.56	100##	6.25	50.0	100	100
12.5	100##	12.5	12.5	12.5	25.0
6.25	100##	6.25	25.0	100##	50.0
3.12	100##	6.25	100##	100##	100##
3.12	100##	100	50.0	50.0	50.0
100##	100##	100##	100##	100##	100##
6.25	100##	3.12	50.0	100	100##
25.0	100##	3.12	50.0	100##	100##
3.12	1.56	100##	25.0	100	50.0
25.0	100##	100	100##	100##	100##
3.12	100##	12.5	100	50.0	12.5
12.5	100##	100##	100##	100##	100##
6.25	100##	6.25	50.0	100	100

APPENDIX II

INSTRUCTIONS FOR PREPARATION OF ALBAMYCIN DRY SENSITIVITY DISCS

APPENDIX II

INSTRUCTIONS FOR PREPARATION OF ALBAMYCIN DRY SENSITIVITY DISCS

A. Materials and Equipment.[1]

Capillary pipette, 13-696 (Pipette automatic capillary designed by Dr. Davis, Eli Lilly) 0.92 ml per delivery.

Three hundred fifty to four hundred Filter paper discs (Fischer Scientific Co. 9-897 Filter paper disc for the assay of penicillin and other antibiotics) 1/2 inch in diameter.

Four 50 ml beakers.

Four calibrated 1 ml pipettes.

Two calibrated 10 ml pipettes.

Crystalline Albamycin 250 mg. with 5 ml accompanying diluent.

Two pair small thumb forceps (1 pr. sufficient).

One 5 ml Luer lock syringe with long needle (19-20 gauge).

Two to three cotton or gauze swabs moistened with 70% alcohol.

Eight to ten 4-inch square wire meshes with four corners of each bent down to make a platform from each mesh.

Three or four small storage bottles.

Two large calcium chloride desiccators (these do not have to be sterile).

---

[1] All equipment and materials must be sterile and all glassware must be washed and free of chemically active substances.

Sixty ml 0.1 M phosphate buffer solution, pH 7.8. The solution is mixed in the following manner: Add 5.1 ml of M/15 KH_2PO_4 with 54.9 ml of M/15 Na_2HPO_4. pH should be checked electrically.

B. Instructions Proper.

1. Under sterile conditions inject the albamycin diluent (entire quantity) into the ampule.

2. Shake the ampule well until all crystals are dissolved and then withdraw entire contents with the syringe and place them in a sterile 50 ml beaker. This solution is called A with a concentration of 50 mg./ml.

3. Take 0.1 ml of solution A and add to this in a second sterile 50 ml beaker 9.9 ml of the phosphate buffer solution. The resulting solution is called R. Concentration is 500 mcg./ml.

4. In a third sterile 50 ml beaker place 29.7 ml of the phosphate buffer and 0.3 ml of R. This will give 30 ml of solution D, concentration of 50 mcg./ml, which when delivered from the capillary pipette (0.92 ml/delivery) will place 4.6 mcg. Albamycin on each disc.

5. One by one lay out the wire meshes, the main parts of which will be elevated by the bent corners, and place on them with sterile forceps 30-40 discs.

6. Now with a capillary pipette apply one drop to each disc, and then place the mesh in the desiccator. It is not harmful to place the wire meshes one on top of the other so long as sterile technique is preserved.

7. Leave the discs in the desiccator for 48-72 hours. Place the discs in sterile storage bottles. This method will yield 350-400 discs.

APPENDIX III

THE BIOASSAY OF THE ALBAMYCIN DRY SENSITIVITY DISC

APPENDIX III

THE BIOASSAY OF THE ALBAMYCIN DRY SENSITIVITY DISC

The disc prepared and dried as indicated is placed in 2.0 ml of BHI broth for one hour. One ml is then withdrawn with a pipette and serial dilutions are made with the test organism Strep 8668. The control indicated inhibition of growth at 0.39 mcg./ml. Inhibition with the broth from which the disc agent had diffused occurred in the 1:4 dilution. Since the dilution was 1:4 and the original sample was immersed in 2.0 ml of broth, the final antibiotic quantity was arrived at by multiplying 4 x 2 x 0.39, which comes to 3.12 mcg./disc. This procedure was repeated twelve times and the results were approximately the same. Similar results were obtained from cutting the disc up with scissors and immersing the fragments in the broth.

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