JCI The Journal of Clinical Investigation

ABSTRACTS

J Clin Invest. 1949;28(5):1039-1055. https://doi.org/10.1172/JCI102137.

Research Article

ABSTRACTS

Penicillin

Factors Affecting Paper Chromatography of Penicillins. MARVIN J. JOHNSON. Department of Biochemistry, University of Wisconsin, Madison, Wisc.

Although penicillin chromatography has been used to illustrate the factors discussed, much of the material is equally applicable to chromatography of other antibiotics.

The performance of an analytical chromatogram may be expressed in terms of its resolving power which in turn may be defined as being inversely proportional to the percentage difference in distribution coefficient necessary to produce a given separation of two components. The resolving power of a chromatogram for any pair of components is calculable if certain basic data are at hand. In other words, it is possible to predict whether a given set of chromatographic conditions will effectively separate a given pair of components, or at least to predict under what conditions separation is most likely to occur.

Variation in pH constitutes a convenient method of changing the rate of migration of all components by an equal factor. Change of solvent usually produces an analogous effect. By proper choice of pH and solvent, any desired component may be brought to the region of maximum resolution in the chromatogram. Variation in amount of mobile phase allowed to flow causes a change not only in the position of the components on the chromatogram, but also in their degree of separation. By changing both pH and amount of solvent flow, enhanced resolution may be obtained. Unless excessive flow rates or other faulty techniques are used, the number of theoretical plates realized per unit length of column is probably largely determined by the type of paper chosen.

Comparative Penicillin Assays. D. K. KITCHEN, E. W. THOMAS, C. R. REIN, and W. E. CRUTCHFIELD, JR. Bristol Laboratories, Inc., New York, N. Y.

The purpose of this study was to establish, from a practical standpoint, the degree of accuracy and consistency inherent in two assay procedures. Known standards of crystalline Penicillin G in three concentrations (approximately 3.0, 0.3 and 0.03 units/cc.) in three diluents (gelatine, phosphate buffer and human serum) were prepared. These were assayed by the "cup plate method" using *Sarcina lutea* as test organism and the "serial dilution method" using *B. subtilis* as test organism. Fifteen duplicates were performed using each method at each concentration. All assays were performed immediately following the preparation of the standards from the dry stable salt. The results are graphically portrayed in a table giving numerical values obtained on average blood levels and the percentage of standard measured by each method. A chart depicting individual assay distribution points has been prepared. This demonstrates clearly that under the conditions studied it is apparent that the "cup plate" assay procedure is more accurate than the "serial dilution" method.

The Pathogenesis and Pathology of Airborne Mouse Pneumonitis Virus Infection in Mice. II. The Effect of Penicillin and Sulfadiazine on the Developing Lesion. CLAYTON G. LOOSLI. Department of Medicine, University of Chicago, Chicago, Ill.

Fatal pulmonary infections can be produced in mice by allowing them to breathe air for one hour in a 60-liter chamber into which is atomized 4 cc. or more of 10^{-1} dilution of mouse lung suspension of mouse pneumonitis virus. Animals die of extensive pulmonary consolidation from ten to 16 days following exposure. Intranasal inoculation (0.05 cc. of virus suspension) kills mice in 48 hours. There is evidence microscopically that the virus vesicle develops extracellularly on the surface of the alveolar walls as well as intracellularly in the bronchial epithelial cells.

Total daily doses for 16 days of 2,000 units of Penicillin G in saline given subcutaneously (500 units per 0.1 cc. dose) immediately before or after airborne inoculation suppressed the development of the lesion but did not sterilize the lungs. Seventy-two hours after discontinuing penicillin, gross lesions were present and large amounts of virus could be obtained from the lungs of the treated animals. Sulfadiazine (8 mgm. per day in four doses, 0.1 cc. subcutaneously) sterilized the lungs after seven days of treatment.

Penicillin G (8,000 units per day in 8 doses, 0.1 cc. subcutaneously) given at three-hour intervals following intranasal inoculation altered greatly the developing virus vesicle as seen microscopically. Sulfadiazine (20 mgm. per day) prevents the development of the virus vesicle.

The above observations on the effect of antibiotics on the mouse pneumonitis virus may be of value in further elucidating the fundamental nature of the viruses of the *psittacosis lymphogranuloma* group. Detailed reports of the above studies will appear later.

Hypersensitivity to Penicillin. J. F. WALDO and JEANNE T. TYSON. Department of Internal Medicine, College of Medicine, University of Utah, and Salt Lake General Hospital, Salt Lake City, Utah.

In certain instances it has been possible to demonstrate by passive transfer, employing the Prausnitz-Kustner technique, a circulating antibody to penicillin. This passive transfer was best accomplished from severely reacting subjects during the most active phase of the reaction. The antigen employed for the passive transfer was crystalline Penicillin G dissolved in saline.

By binding crystalline benzyl penicillin (Penicillin G) to pure human albumin and injecting this in rabbits an antibody has been produced which gives a moderately strong complement fixation reaction with the albumin-penicillin mixture after all reactivity to human albumin has been absorbed. Penicillin alone, administered to the rabbit, has failed to produce such an antibody. Some evidence has also been obtained which indicates that when penicillin is bound to human albumin, the resulting anti-

genic product behaves in the manner of a mixed antigen while the human albumin used in the experiment behaves in the manner of a pure antigen. Quantitative studies of this antigen-antibody system seem to substantiate this.

Comparative studies with n-heptyl penicillin (Penicillin K) are now in progress because it is believed by most observers that this penicillin is more completely bound to the protein than is benzyl penicillin. If this is true the antibody reaction should be more marked.

Inasmuch as it is known that penicillin is bound *in vivo* to albumin it seems reasonable to suppose that when penicillin is administered to man, the penicillin haptene coupled to albumin *in vivo* constitutes an antigen which leads to the production of an antibody specific for this haptene group. This might well account for the penicillin sensitivity reactions observed in man.

The Jarisch-Herxheimer Reaction in Early Congenital Syphilis. A Study of 93 Patients Treated with Penicillin Alone. HAROLD A. TUCKER[1] and OSVALDO A. PARDO.[2] U. S. Public Health Service and the Johns Hopkins Venereal Disease Research and Post-Graduate Training Center, Baltimore, Md.

Between 1943 and March, 1948, 93 children under three years of age were diagnosed as having untreated early congenital syphilis at the Johns Hopkins Hospital. Each was treated with penicillin as the sole antisyphilitic agent. A fever of 38° C. or greater in a previously afebrile patient, occurring from four to 36 hours after the start of penicillin administration and lasting less than 36 hours, was interpreted as a febrile Jarisch-Herxheimer reaction. Forty-five children (48.5 per cent) showed such reactions.

No relationship between incidence and magnitude of the reaction and age, race, sex, type of congenital syphilis, serologic titer or outcome, was observed. The type of penicillin (*e.g.*, amorphous or crystalline G) likewise did not appear to be a factor nor, within the range of 250 to 7,690 units/kg. of body weight, did the gravimetric dosage given. When it occurred, the reaction seemed to be entirely analogous to that seen in the adult with early acquired syphilis.

Five of the 93 children died and in each case prematurity, malnutrition, general debility and/or intercurrent disease were complicating factors. None of these deaths was believed by us to be directly attributable to the Jarisch-Herxheimer reaction. It was our feeling that the outcome was largely dependent upon the general condition of the patient, and that a successful result depended as much, or more, on general pediatric management as upon the type or amount of syphilotherapy employed.

Treatment of Various Infections with a Single Injection or Injections at Five Day Intervals of 300,000 Units (1 cc.) Procaine Penicillin in Oil with 2 per cent W/V Aluminum Monostearate. WALTER KURLAND and HAROLD L. HIRSH. Georgetown University Medical Division, Gallinger Municipal Hospital, and the Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

The present study was undertaken to determine whether common mild and moderate infections can be adequately treated by a single 1 cc. dose of procaine penicillin in oil with aluminum monostearate containing 300,000 units.

Included in this series were 41 patients with scarlet fever. In all but three, throat cultures became negative within 48 hours after administration of the penicillin. These three became negative after 72 hours. One of the three became positive again after 96 hours and persisted as a carrier until a second dose was given on the 11th day, whereupon he again became negative. Two of the 41 patients developed complications. One developed a serous meningitis on the seventh day which cleared rapidly within several days. Another patient developed a purulent otitis media on the tenth day, after having been taken home inadvertently on the preceding day by her parents. Temperatures fell to within normal limits within 48 to 72 hours in all patients except three, and in two additional patients therapy was given. It was strongly suspected that one of these patients did not receive his initial dose and the other required sulfonamides.

Of the 82 patients with lobar pneumonia, three failed to respond adequately to the single injection but recovered following the administration of additional doses, and in two suppuration in the pneumonic area developed which was treated with intensive antibiotic and chemotherapeutic drugs. Seven patients, all with serious complications, died: delirium tremens (2); severe cardiac disease (2); diabetic acidosis (1); asthma with purulent bronchitis and an acute lung abscess at another site (1); and possible renal disease (1).

Successful results were obtained in nine patients with otitis media; one with a hemolytic streptococcus pharyngitis; one with a Vincent's infection of the throat, and three with cellulitis.

Delayed Administration of Oral Penicillin[1] *as Prophylaxis for Gonorrhea.* V. W. H. CAMPBELL,[2] WILLIAM J. DOUGHERTY,[2] and C. E. CURTIS.[3] Communicable Disease Control Section, Department of the Navy, Washington, D. C.

The study of the prevention of gonorrhea by Eagle *et al.* has indicated that the utilization of per oral penicillin, 100,000 to 250,000 units, within a short time after venereal exposure is highly effective in the prevention of gonorrhea.

The operation of a Naval Force in an area where high venereal disease rates prevailed afforded the opportunity to conduct further study designed to determine the feasibility and efficiency of prophylaxis by oral penicillin given 12 to 24 hours after exposure to venereal disease.

[1] Department of Bacteriology, University of Southern California School of Medicine, Los Angeles, California.

[2] Department of Dermatology and Syphilology, University of Habana, Cuba.

[1] The penicillin tablets and placebos used in this study were furnished by Commercial Solvents Corporation.

[2] Commander, MC, USN.

[3] Lieutenant, MSC, USN.

Two thousand and eighty men participated in the study. One thousand and fifty-nine men in the experimental group experienced 1,833 liberties and 2,625 exposures, resulting in nine cases. This yielded a rate of 4.75 cases per 1,000 liberties and 3.4 cases per 1,000 exposures.

One thousand and twenty-one men in the control group experienced 1,891 liberties and 2,686 exposures, resulting in 22 cases of gonorrhea. This yielded a rate of 11.6 cases per 1,000 liberties and 8.2 per 1,000 exposures.

The experimental group received 200,000 units of sodium Penicillin G by mouth following a mean elapsed interval between exposure and penicillin prophylaxis of 15 hours. The variation about this mean was approximately 3.5 hours.

Contrasted to the study reported by Eagle *et al.*, in which the probable elapsed time of two hours between exposure and per oral penicillin was reported, this study indicates that within an increasing interval between exposure and utilization of the tablets, the prophylaxis efficiency of per oral penicillin is reduced.

Use of Penicillin in Oral Vincent's Infection as a Means of Rapidly Controlling "Epidemics." J. Looby[1] and Carl A. Schlack.[2] Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

This project (X-345) was approved by the Chief of the Bureau of Medicine and Surgery, U. S. Navy, 15 March 1944. Oral color photographs were used as controls and, in all, 107 patients were treated. Fifty-two patients were given 25,000 units of penicillin intramuscularly every three hours until 100,000 to 200,000 units had been injected. Fifty-five patients received topical application of 250 units per cc. saline solution; 10 cc. sprayed on tissue and 3 cc. swabbed on lesions (swab remained in mouth for 10–20 minutes). No other form of treatment was employed (no scaling, mouth washes, oral hygiene, change of habits [dietary or otherwise]). Of those receiving intramuscular injections, 50 showed disappearance of painful symptoms and improvement in tissue appearance in 24 to 48 hours; one reached this stage in 72 hours, and one was indeterminate. Of the groups receiving topical application, 44 showed improvement in 24 to 72 hours, nine were indeterminate, and two failed to improve. The majority in both groups showed reduction in bacteriologic smear positives for Vincent's spirochetes and fusiform bacilli after treatment.

It appeared that the intramuscular injection of penicillin was more effective than the topical application as a means of rapidly controlling "epidemics" of oral fusospirochetosis (Vincent's infection).

Further Observations on Protection against the Lethal Effects of Bacterial Endotoxin by Penicillin and its Impurities. Walter D. Hawk, William H. Anderson, and C. Phillip Miller with the technical assistance of Barbara Bowden. Department of Medicine, University of Chicago, Chicago, Ill.

Previous findings regarding protection of mice against the lethal effect of certain bacterial toxins by the intraperitoneal administration of penicillin and its impurities have been enlarged and several related questions have been answered.

1. This means of protection, which has proved effective against the endotoxins of meningococcus, gonococcus, *Salmonella aertrycke,* and a number of other Gram-negative organisms, has been shown to be without effect against exotoxins obtained from Staphylococcus, *Cl. botulinum* and *Cl. tetani.*
2. Administration of a number of drugs hitherto untested (streptomycin, aureomycin, the anti-histaminics, etc.) has provided none of the protection shown with penicillin.
3. Preliminary administration of sublethal doses of homologous endotoxin (as described by Cantoni) provides only slight protection as compared with that obtained with impure penicillin. Colchicine with penicillin provides no protection.
4. The administration of adrenal cortical extract with penicillin or adrenal cortico-trophic hormone with penicillin provides only slight protection.
5. The intravenous administration of penicillin and its impurities is of some benefit but cannot be carried out satisfactorily because of the toxicity of the drug in its present unpurified state.
6. Several new means of preparing the "penicillin impurities" have been tested. It has been shown that the protective factor(s) in this material is readily obtained by extraction of the "spent beer" discarded after commercial extraction of penicillin from the fermented liquor.
7. A rapid and economical method of bio-assay of the protective factor has been developed.
8. The "penicillin impurities" used in this work have been shown regularly to contain large amounts of the "enhancement factor" described by Welch, Hobby and others. Unusual prolongation of penicillin blood levels following the administration of these materials could not be demonstrated in our experiments.

Certain Effects of Antibiotics on Animal Tissue Homogenates and Subcellular Elements. Kent Wight and Dean Burk. National Cancer Institute, Bethesda, Md.

These studies were carried out using aerobic methylene blue reduction and Warburg techniques. For the methylene blue reduction studies, mouse livers were homogenized with a mortar and pestle in 0.01 M phosphate. The substrates were malate, pyruvate, acetate, lactate, alcohol, oxalacetate, fumarate, and citrate at 0.01 M and in each case 4 ppm methylene blue. Purified penichromin, a yellow component of crude penicillin, increased the reduction time 100 per cent above that of the control. With malate present, the effect was even more striking. Penicillin and streptomycin increased the reduction time as much as 50 per cent in concentrations of 1,000 ppm or higher. The

[1] Commander, DC, USNR (inactive).

[2] Commander, DC, USN.

same effects were observed with acetate and in the case of streptomycin also with ethyl alcohol. For pyruvate none of the antibiotics inhibited methylene blue reduction. Other antibiotics studied were subtilin and siderophilin.

The tumors used were the S-91a grown on C mice and the C_3HBA on C_3H mice. The time of reduction of methylene blue by the S-91a tumor fractions was one-fifth the time required by liver homogenates. This was further affected by penichromin (> 1,000 ppm) to the extent of 100 per cent.

The particulate fractions were prepared by homogenizing the tumors in 0.85 per cent saline, filtering through glass wool, and centrifuging at 26g for separation of the nuclei followed by 9,000g for the mitochondria.

In the case of the S-91a tumor homogenates, the manometric studies showed inhibition of succinate oxidation by penichromin at 500 ppm of 50 per cent and no effect by actidione, aspergillic acid, or subtilin.

For the mitochondrial fraction using the C_3HBA tumor in the presence of cobalt at 50 and 20 ppm, the Q_{O_2} of 4.6 was decreased to 1.4 and 3.1 respectively. Similar results were obtained with penichromin.

Thus some antibiotics have a striking effect on the enzymes of animal tissues at the subcellular level while some do not except at very high concentrations.

Studies on Bromine-Oxidizable Sulfur-Containing Compounds in Mold Metabolism. CLAUDE H. PLUMLEE and ARTHUR L. POLLARD. Department of Bacteriology, The University of Tennessee, Knoxville, Tenn.

In the course of a quantitative study of the sulfur metabolism of *Penicillium chrysogenum* Q-176 it was found that sulfur compounds oxidizable to sulfate by bromine water were formed and that they accounted for over one-third of the organic sulfur in the culture filtrate. The organism was grown on Czapek-Dox medium containing sulfur only in the form of magnesium sulfate. It was certain, therefore, that all forms of organic sulfur were synthesized by the organism. Other investigators have found that the sulfur in thiamine, biotin, cysteine, cystine, and glutathione is not oxidizable to sulfate by bromine water. Apparently this oxidation is confined to very specific configurations of the molecule such as are found in the xanthogenic acids and in thioacetamide. It is not clear whether such compounds are intermediates in the process of penicillin synthesis or whether they are formed in equilibrium with other sulfur compounds, one of which is penicillin.

The subject is of considerable interest because of the great number of physiologically active sulfur compounds and the wide variation of activity with molecular configuration. Thioacetamide, for example, has been shown to be readily utilized by *Aspergillus niger* whereas some of the xanthates, thiuram derivatives and disulfids are highly fungistatic.

Streptomycin

The Disappearance of Streptomycin-Resistant Bacteria During or After Streptomycin Therapy. MORTON HAMBURGER and JEROME R. BERMAN. Department of Internal Medicine, University of Cincinnati, Cincinnati, Ohio.

Though the appearance of streptomycin-resistant bacteria during the course of streptomycin therapy has been abundantly demonstrated, less attention has been paid to the ultimate fate of these resistant forms. Observations upon this problem have been made during a study of the development of resistant coliform bacilli in the stools of patients given intramuscular streptomycin for tuberculosis or oral streptomycin for intestinal tract infections.

Suspensions of 0.5 cc. stool in 5 cc. saline were streaked on each of two eosin-methylene blue plates, one containing 100 gamma streptomycin per cc. and the other no streptomycin. Colonies were picked from representative plates for more accurate determination of streptomycin sensitivity in a serial dilution test.

In no case were coliform bacilli recovered prior to the administration of streptomycin resistant to more than 2.5 gamma per cc. When streptomycin-resistant forms did appear, 156 to more than 2,500 gamma were necessary for growth inhibition.

In five cases (three treated orally and two intramuscularly) where streptomycin was discontinued after the appearance of highly resistant variants, the resistant strains were replaced by sensitive forms. This process started very soon after the cessation of treatment. Whereas during treatment resistant forms were recovered from both the eosin-methylene blue plate containing streptomycin and the plate which contained none, these forms practically disappeared from the latter after treatment. Days or weeks later, even the plate containing streptomycin failed to show growth when stool suspensions were streaked upon it.

In two cases of tuberculous peritonitis where improvement occurred during treatment, the resistant variants disappeared while the patients were still receiving streptomycin.

Evaluation of Oral Streptomycin in Specific Enteritis in Infants and Children. SIDNEY ROSS, E. CLARENCE RICE, FREDERIC G. BURKE, and JOHN A. WASHINGTON. Children's Hospital, Washington, D. C.

Thirty-four cases of shigella enteritis were treated with oral streptomycin. Twenty-five of the cases were acute while nine were carriers. The average dose of streptomycin orally was 400 mgms. every four hours. The duration of treatment ranged from seven to 19 days with an average of 10.7 days per patient. In the acute cases the positive stool cultures became negative rapidly generally within an average of one to two days. In the carrier group none of the 12 cases showed positive stool cultures within 24 hours following initiation of therapy. An average of 11 negative stool cultures was obtained on each patient before he was considered cured. Once the stool culture became negative, no cultural reversals were noted during the hospital stay. In five of the cases, however, there was a recurrence of positive stool cultures within one month after discharge from the hospital.

Clinically, the improvement was commensurate with the salutary effect observed bacteriologically.

Eight cases of salmonella enteritis in infants and children were treated with streptomycin in large doses ranging from 2.4 to 3.6 gms. per day. Three of the patients received the drug orally only while the other five were given the antibiotic both orally and intramuscularly. In spite of the demonstrated sensitivity of the organism to streptomycin *in vitro* in each case, only one of the eight cases was rendered permanently free of the organisms. In the other seven cases an inhibitory effect on both the salmonella organism and the normal stool flora was observed during the period of drug administration; however, within three to eight days after streptomycin was discontinued, the pathogen reappeared in the stools. No significant increase of streptomycin resistance during the course of therapy was noted in any case.

The Treatment of Tuberculosis with Small Doses of Streptomycin. BERNARD MILLOFF, SOL KATZ, and HAROLD L. HIRSH. Georgetown Medical and Tuberculosis Divisions, Gallinger Municipal Hospital, and the Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

Although the value of streptomycin in the treatment of tuberculosis has been rather fully established, a complete appraisal of dosage and duration of treatment has not been ascertained. Therefore, we have treated a variety of tuberculous infections with small doses of streptomycin. All patients received one-half gram per day in one dose except four with miliary tuberculosis who were given one-half gram twice a day. This dose schedule was selected on the supposition that with the smaller doses the development of organism resistance is not increased and streptomycin toxicity is decreased.

The following results were obtained:

Miliary—5—all developed meningitis and died;
Bronchial—4—favorable response after prolonged therapy;
Pericarditis—2—one favorable response, one failure;
Pleurisy with effusion—1—no response;
Peritonitis—2—relapse after improvement;
Mediastinitis—1—favorable response;
Adenitis—3—no response;
Osseous—4—no response;
Draining sinuses—7—apparently healed.

In two patients with tuberculous bursitis incision and drainings while under streptomycin therapy resulted in healing.

In forty-two patients with 80 operations, which included thoracoplasty, lobectomy and pneumonectomy, no tuberculous spreads occurred with the use of one-half gram per day in a single injection for seven days preoperatively and post-operatively.

Streptomycin in doses of one-half gram per day would appear to be definitely effective in the treatment of bronchial tuberculosis and draining sinuses. It would also be recommended that this same dosage be used in the prevention of tuberculous spreads in the surgery of tuberculous patients. In those tuberculous infections where streptomycin is generally effective, smaller doses are apparently as good.

Incidence of Vestibular Dysfunction Following Intramuscular Administration of Various Doses of Dihydrostreptomycin. SOL KATZ, WALTER KURLAND, and HAROLD L. HIRSH. Georgetown Medical and Tuberculosis Divisions, Gallinger Municipal Hospital, and the Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

Recently a reduced streptomycin compound, dihydrostreptomycin, has been developed which appears to be less neurotoxic in preliminary studies in animals and humans.

On the basis of these data a study of dihydrostreptomycin was undertaken. Included in our series are 26 patients. Five patients received 1 gram per day in divided doses every six hours and none have developed evidences of vestibular dysfunction after 13 weeks of therapy. However, of three patients on 0.5 gram every six hours (2 grams per day) for 13 weeks one showed vestibular dysfunction after the sixth week of therapy and the other after the ninth week.

Doses of 3 grams per day (0.75 gram every six hours) were administered in eight patients. Three were treated for 13 weeks and one for 60 days without evidences of impairment of vestibular function. Two demonstrated impairment after six and eight weeks of treatment, respectively. In one, vestibular dysfunction appeared after the first treatment week and in another, a 13 year old boy, after the seventh week.

Three patients were given 3 grams per day in doses of 1.5 grams every 12 hours for a period of four to five weeks. Two developed vestibular impairment after the first and second weeks of therapy, respectively. One was 69 years of age and the other had unilateral dysfunction from a previous course of 150 grams streptomycin. One patient received 1 gram of dihydrostreptomycin for 14 days followed by 3 grams for 18 days. He showed no impairment of vestibular function.

Five patients received 1 gram every six hours for 7, 35, 35, 60 and 60 days, respectively, without impairment of vestibular function. Vestibular dysfunction was noted in one patient on this dosage-schedule after the fourth week of therapy.

The Therapeutic Use of Antibiotics: Comparative Activities, Synergism, and Resistance

The Summation of Penicillin and Streptomycin Activity In Vitro and in the Treatment of Subacute Bacterial Endocarditis. WILLIAM C. ROBBINS and RALPH TOMPSETT. Department of Medicine of the New York Hospital-Cornell University Medical College, New York, N. Y.

Despite the fact that the summation of action of two drugs is an established principle of pharmacology, few examples of unquestionable summation of the action of

two antimicrobial agents in the treatment of infection in man are to be found.

The therapeutic effectiveness of penicillin in subacute bacterial endocarditis has resulted in the arrest of the infection in all but 10 per cent of cases. The majority of this latter group of patients are infected with Streptococci of the Lancefield serologic Group D, the most important members of which are commonly termed *Streptococcus fecalis* and *Streptococcus zymogenes*. These enterococci are highly insensitive to penicillin, and although temporary suppression of the endocarditis caused by these organisms is readily attained with its administration, relapse almost invariably ensues after the penicillin is discontinued. Enterococci also exhibit considerable resistance to streptomycin, and results of the treatment of enterococcal endocarditis with streptomycin, though of limited extent, have been only slightly better than those achieved with penicillin.

A study has been made of the individual and combined actions of penicillin and streptomycin *in vitro* on strains of *Enterococcus* and *Streptococcus viridans* isolated from patients with endocarditis. It has been observed that the two drugs act as coadjuvants in inhibiting growth of all strains studied, total summation of partial effects occurring throughout a wide range of concentrations of each constituent drug. For example, complete inhibition of growth was achieved with a mixture containing one-fourth the inhibitory concentration of penicillin plus three-fourths of the inhibitory concentration of streptomycin, or with a mixture containing one-half the inhibitory concentration of each. In some instances inhibition has occurred with constituent drug fractions which total less than one, indicating an effect greater than summation.

Six patients with persistent enterococcal bacteremia, five of whom had endocarditis, have received six million units of penicillin and 2 grams of streptomycin, or its dihydro derivative, daily. Two patients received the combined therapy only terminally, but four completed a course of four to eight weeks of this therapy. Bacteremia was promptly reversed and there was sustained clinical improvement in all four. All but one patient have been followed for periods of from six to 12 months and none has relapsed.

The uniformly good results with these patients have been in striking contrast with the previous experience with the use of either drug alone in these infections, and suggest the operation of a summation effective *in vivo* comparable to that observed *in vitro*. Experiments are now in progress which indicate that the summation of penicillin and streptomycin may apply to organisms other than penicillin-resistant streptococci, and that summation may occur with other antibiotic combinations. The use of combinations of antimicrobial drugs may offer an effective method of achieving permanent arrest of infections which can now only be suppressed by the use of one drug alone.

Pharmacological Studies on Actidione. ANDRES GOTH and FABIAN J. ROBINSON. Department of Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas.

Actidione is characterized by a marked growth inhibitory activity against certain fungi and by a considerable species variation in its toxicity to mammals. The purpose of the present study was twofold: to demonstrate inhibitory blood levels in various species of mammals following the injection of actidione and to study the nature of its toxicity.

A solution of crystalline actidione was injected intraperitoneally into rats, guinea pigs, and dogs. The citrated plasma of these animals was tested by a serial dilution method in tryptosephosphate broth for inhibitory activity against *Saccharomyces pastorianus* ATCC2366.

After the injection of 1 to 10 mg. of actidione per kg. into rats, 5 to 20 dilution units were detected in the plasma in 30 minutes. The inhibitory activity decreased somewhat in 90 minutes. The results were essentially the same in guinea pigs and dogs.

It appears from these data that actidione is absorbed rapidly from the intraperitoneal site and is not rapidly inactivated in the body.

The injection of as little as 1 mg. of actidione per kg. body weight intravenously into the dog is followed by vomiting in a few minutes. This emetic effect is prevented by nembutal anesthesia. Nausea has also been observed in humans following the injection of actidione.

Comparisons of Six New Antibiotic Agents in Experimental Infections in Mice. ELEANOR A. BLISS and PATRICIA TODD. Department of Preventive Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md.

The therapeutic activities of penicillin O, aureomycin, chloramphenicol, polymyxin D, polymyxin B and Q 19 (circulin) were compared in mouse infections induced with a type I pneumococcus, a Group A hemolytic streptococcus, and a strain of *K. pneumoniae*, type A. Penicillin G and streptomycin were included as standards. Two strains of mice were employed, a mixed strain of Swiss mouse and the pure CF_1 strain. Therapy was administered by the subcutaneous route, three treatments being given at 0, 5½, and 23 hours after infection. Two or more agents were compared in each experiment, usually at three doses each. The median protective dose was estimated.

The polymyxins and Q 19 were most effective in the therapy of the *K. pneumoniae* infection; the PD_{50} for all three was less than 1 mg/kilo per treatment. The PD_{50} for streptomycin was 2.5 mg/kilo; for aureomycin and chloramphenicol it was 35 to 50 mg/kilo. In the treatment of the streptococcal infection, Penicillin G and O were most effective with PD_{50}'s of about 0.3 mg/kilo. The PD_{50} of aureomycin was 4 mg/kilo while that of streptomycin was 70. The PD_{50} for chloramphenicol here and in the pneumococcal infection could not be determined because of the limited solubility of the agent, even in 20 per cent alcohol. It was above 80 mg/kilo. In the pneumococcal infection the same order of activity pre-

vailed, with the notable exception that aureomycin was the most effective agent in the CF_1 mice. The PD_{50} for Penicillin G in this mouse was ten times as high as in the mixed strain of mice.

The Effect of Antibiotics on the Growth of Histoplasma Capsulatum in Vitro. BEN D. CHINN and MARIO MOLLARI. Department of Bacteriology and Preventive Medicine, Georgetown University School of Medicine, Washington, D. C.

A study was made of the effect of several antibiotics on the growth of *Histoplasma capsulatum in vitro*. *H. capsulatum* is the etiological agent of Histoplasmosis, a disease characterized by emaciation, leukopenia, anemia and irregular pyrexia, and produces cutaneous ulcers and primary involvement of the lungs. The antibiotics used included penicillin, streptomycin, bacitracin, aureomycin, polymyxin A, aerosporin (polymyxin B) and chloromycetin.

The measure of activity against the test organism was determined by the streak method, the agar diffusion method, and the serial dilution method. Penicillin showed inhibitory effects in concentrations above 5,000 units/ml of medium (blood agar). A more marked effect was produced by streptomycin. Inhibition of growth began with 50 mg/ml of medium and only slight growth occurred beyond 200 mg/ml. Chloromycetin showed no antibiotic activity. Aureomycin and polymyxin A showed slight activity above concentrations of 20 mg/ml, but increases in the concentration beyond this amount did not produce a corresponding effect on the growth of the test organism. Bacitracin appeared to have a marked inhibitory effect on the growth of the organism in concentrations of 600 units or higher. Aerosporin had an activity similar to bacitracin, its antibiotic activity beginning with 5 mg/ml.

Among the antibiotics tested streptomycin appeared to have the greatest inhibitory action on *H. capsulatum*. The effect of some of these antibiotics in experimentally infected animals is under investigation.

The treatment of certain forms of Histoplasmosis has been very unsatisfactory; the disease is generally considered highly fatal. The possibility of antibiotic therapy appears worthy of consideration.

The Combined Action of Antibiotics and Sulfonamides in Experimental Infections. C. W. PRICE, W. A. RANDALL, and H. WELCH. Federal Security Agency, Food and Drug Administration, Washington, D. C.

Various combinations of penicillin, streptomycin, aureomycin, chloramphenicol, bacitracin, polymyxin, sulfanilamide, sulfadiazine, sulfathiazole and sulfamerazine were tested *in vivo* for possible synergistic action, using *Streptococcus hemolyticus* C 203 Mv as a test organism. In these experiments organisms were injected with and without mucin. When mucin was used the virulence of the streptococci was markedly increased; relatively larger doses of the antibacterial agents were required and changes in the ratios of combinations showing a synergistic effect resulted. The results obtained in experimental infection emphasize the difficulty of translating experimental observations into terms of clinical effectiveness.

Alteration and Inhibition of Sea-Urchin Egg Division by Antibiotics. IVOR CORNMAN. Sloan-Kettering Institute for Cancer Research, New York, N. Y.

As a part of a cancer chemotherapy screening program, sea-urchin eggs are being used to detect substances which inhibit cell division and in the study of their effects on mitosis. The eggs of *Arbacia* and *Lytechinus* are exposed ten minutes after fertilization. Normally, cleavage follows at 50–60 minutes after fertilization at 23°–26° C. Divided eggs are counted at intervals to determine inhibition or retardation of cleavage, and such abnormalities as are visible in the living egg are recorded.

Crude penicillin has received special attention because of its ability selectively to kill tumor cells and because Burk has found it to contain a potent metabolic inhibitor. Older pharmaceutical grades of penicillin retarded cleavage at about 300 mg/L. A series of five penicillins supplied by Burk proved to have relative potencies of 1 : 2 : 200 (three having the middle rating) compared with respiratory inhibition ratings of 1 : 10 : 300. Burk's partially purified *Penichromin* was effective at 5 mg/L, while purified penicillin required 1 g/L to retard cleavage.

A series of filtrates from *Aspergillus* cultured under a variety of conditions were nearly all effective in retarding cleavage. Gliotoxin displayed a distinctly different pattern of action, quickly destroying the eggs when used at concentrations sufficient to delay cleavage (2 mg/L). Percentages of eggs completing second cleavage were: 100 per cent, 30 per cent, and 0 per cent at 1, 2, and 4 mg/L.

Clavacin is another of the more active purified antibiotics, inhibiting all cleavage at 5 mg/L. It was, therefore, more active than its component parts tested separately: the pyrone ring and the five-membered lactone ring.

Search for New Antibiotics

The Isolation and Purification of Neomycin. E. AUGUSTUS SWART, SELMAN A. WAKSMAN, and DORRIS HUTCHISON. New Jersey Agricultural Experiment Station, Department of Microbiology, Rutgers University, New Brunswick, N. J.

Neomycin has been isolated from culture filtrates of *Streptomyces* 3535 by the following sequence of steps: acidification to pH 2.0–2.5, clarification with activated charcoal, neutralization, adsorption on decalso, elution with 10 per cent ammonium chloride, adsorption on activated charcoal from pooled ammonium chloride eluates at pH 7.0, elution by 0.05 N hydrochloric acid in 50 per cent methanol, neutralization by passage through an amberlite IR-4B column or by addition of aqueous sodium hydroxide, concentration *"in vacuo,"* at 30–35° C, to 0.01–0.1 of original volume, picking up residue in methanol and precipitating with acetone, or picking up residue in water and lyophilizing. The concentrates of neomycin

chloride showed an average potency of 100 units/mg, some solids ranging as high as 225 u/mg.

Chromatography on columns of Darco G-60 or alumina, using methanol as solvent and eluant, has yielded more highly potent concentrates. Precipitation with picric acid, followed by direct conversion to the hydrochloride, has also given more potent solids.

Another antibiotic, having the same antibacterial spectrum as neomycin but having antifungal activity as well, has been isolated from the culture filtrate. Most of this material was removed by the preliminary acidification of the broth and by treatment with Norit H15, leaving neomycin in the filtrate. Norit A, Amberlite IR-100-H, and decalso adsorbed neomycin from culture filtrate, leaving the second agent in the filtrate.

Neomycin is soluble in water, slightly soluble in methanol, and insoluble in other organic solvents. It is stable at pH 2.1 for one hour at 100° C, four hours at 25° C, and five days at 5° C. A negative Sakaguchi test indicates the absence of mono-substituted guanidine groups. The maximum in the distribution curve occurs at tube 21 when neomycin is distributed between pentasol and borate buffer at pH 7.6, using stearic acid as a carrier, in the 24 plate Craig Counter-current Distribution Machine. Streptomycin and streptothricin show respective peaks, in the same system, at tubes 9 and 8.

Borrelidin, a New Antibiotic with High Anti-Borrelia Activity and Penicillin Enhancement Properties, Produced by a Streptomyces Species. J. BERGER and M. W. GOLDBERG. Research Laboratories of Hoffmann-La Roche, Inc., Nutley, N. J.

An antibiotic with unusual specificity and high activity has been found to be produced by several actinomycetes. It is highly active *in vitro* against *Sarcina lutea* and certain micrococci but relatively inactive against other common test bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis.* The growth of a protozoan, *Tetrahymena geleii,* is inhibited at a dilution of 1:200,000. As little as 0.0001 mg. per ml. can be detected by the cylinder plate technique when *S. lutea* is used as test organism. The substance has been isolated in crystalline form (L. M. Jampolsky and M. W. Goldberg) and was named "Borrelidin" because of its outstanding activity *in vivo* against *Borrelia* species (R. J. Schnitzer *et al.*).

The culture producing the best yield of borrelidin is a new *Streptomyces* species. It grows readily on complex organic media, but only sparingly on synthetic liquid media. Under usual methods of handling, the culture yields strains which vary widely in appearance and antibiotic production. Borrelidin is produced by the *Streptomyces* in a variety of liquid media, in stationary shallow layers or in aerated submerged culture. Particularly suitable are media containing soybean meal, glucose, and phosphate.

Small amounts of borrelidin-like substances have been found in sources of penicillin enhancement factors, such as inactivated impure penicillin, clarase enzyme preparations and even in corn steep liquor. In spite of the fact that pure borrelidin does enhance penicillin activity *in vivo* (E. Grunberg *et al.*), no claim can be made for its identity with any of the postulated penicillin enhancement factors since certain differences have also been observed.

Isolation of Crystalline Borrelidin from Streptomyces Broth and of Borrelidin-Like Concentrates from Crude Penicillin. L. M. JAMPOLSKY and M. W. GOLDBERG. Research Laboratories of Hoffmann-La Roche, Inc., Nutley, N. J.

A new antibiotic, borrelidin, has been isolated in crystalline form from the culture medium of a new *Streptomyces* species. The antibiotic exhibits an unusual degree of specificity, being highly active only against *Sarcina lutea in vitro* and against several strains of *Borrelia in vivo.* Concentrates exhibiting some of the biological properties of borrelidin were also obtained from crude penicillin and other sources reported to contain penicillin enhancement factors. Borrelidin itself was found to enhance the activity of penicillin in certain experimental infections of mice (E. Grunberg *et al.*).

Borrelidin can be isolated from the culture solution by extraction with butyl acetate. The residue obtained by evaporation of the extract can be purified by treatment with bentonite clay in various solvents and finally, by extraction from ether with alkali. Concentration of a benzene extract of the acidified alkali solution yields crystalline borrelidin. Recrystallization from benzene gives pure borrelidin, m.p. 145–146°, $[\alpha]_D^{27} = -28°$ in ethanol.

Borrelidin is an acid. A crystalline methyl ester (m.p. 153–154°) and a crystalline p-nitrobenzyl ester (m.p. 161°) have been obtained. The methyl ester yields a crystalline diacetyl- (m.p. 190°) and a crystalline di-p-nitrobenzoyl-derivative (m.p. 156–157°). The microanalytical data so far obtained indicate an empirical formula of $C_{28}H_{43}O_6N$ for borrelidin. The ultra-violet absorption spectrum in isopropanol shows a maximum at 256 mμ ($E_{1\text{ cm.}}^{1\%} = 550$), indicating a site of conjugated unsaturation in its molecule.

Enhancement of Penicillin Activity by Borrelidin. E. GRUNBERG, D. ELDRIDGE, and G. Soo-Hoo. Research Laboratories of Hoffmann-La Roche, Inc., Nutley, N. J.

The new antibiotic, borrelidin, the principal chemical and biological properties of which have been described in the preceding abstracts, did not show any demonstrable chemotherapeutic activity *in vivo* if tested in experimental infections with *β-hemolytic streptococci, pneumococci type 1 and 2, Klebsiella pneumoniae, Salmonella schottmuelleri, Eberthella typhosa, Mycobacterium tuberculosis,* strain H37Rv, and *Cryptococcus hominis.* This substance, however, possessed some growth inhibiting effect *in vitro* against the organisms enumerated above.

Attempts were made to show that borrelidin possesses properties of enhancement of crystalline Penicillin G similar to those described for unknown factors obtained by inactivation of impure penicillin (Hobby *et al.*, Dunham

et al., Miller *et al.*, Cole, Burk *et al.*, and Welch *et al.*). It could indeed be demonstrated that the addition of very small amounts of borrelidin increased the effect of crystalline Penicillin G in experimental infections with group A, type 3 hemolytic streptococci. The findings may be summarized by the comparison of the CD50 of Penicillin G in the presence and absence of borrelidin.

Single treatment	CD50 of Penicillin G
Cryst. Penicillin G alone	0.56 mg/kg (935 units/kg) subcut.
Cryst. Penicillin G + borrelidin 0.2–1.7 μg/kg	0.25 mg/kg (417 units/kg) subcut.
Cryst. Penicillin G alone	1.16 mg/kg (1,937 units/kg) per os
Cryst. Penicillin G + borrelidin 3.0 μg/kg	0.71 mg/kg (1,185 units/kg) per os

There was also found a marked trend to enhancement of Penicillin G by borrelidin in experimental rabbit syphilis, in the protection of mice against meningococcal endotoxin, and in the growth inhibition of *Trichomonas vaginalis*. In addition borrelidin inhibited the respiration of staphylococci as tested by the methylene blue method.

We were, however, not able to demonstrate with borrelidin the enhancement of Penicillin G in infections with *E. typhosa* as described by Welch and with type 1 pneumococci as described by Hobby. The enhancement of Penicillin G in infections with *Salmonella schottmuelleri* was also not observed.

No definite statement can be made at the present time regarding the identity of borrelidin and any of the enhancement factors described.

The Anti-borrelia Effect of Borrelidin. R. J. Schnitzer, M. Buck, and A. C. Farr. Research Laboratories of Hoffmann-La Roche, Inc., Nutley, N. J.

During the routine screening in experimental infections of mice it was found that borrelidin, a new antibiotic found and isolated in crystalline form by M. W. Goldberg and his associates, J. Berger and L. M. Jampolsky (see the abstracts of these authors), exerted a striking effect on borrelia infections. This is illustrated by the following table:

Animal	LD50	CD50: *mg/kg*		
		Borrelia tick	*B. Novyi*	*B. Obermeieri*
	mg/kg			
Mice	74.7	0.6	2.0	1.7
Rats	1.78	—	—	0.9

Borrelidin-like substances in concentrates from impure penicillin, clarase and corn steep had a similar activity.

Borrelidin was also effective toward a partially penicillin resistant strain of *B. Novyi*. It was comparatively easy to produce a borrelidin fast strain of *B. Obermeieri* which still responded to As-compounds, Au-compounds, penicillin and aureomycin. This indicates that borrelidin represents a type of anti-borrelia agent different from those mentioned before.

No effect of borrelidin was seen in experimental rabbit syphilis and in trypanosomal infections. The compound exerted, however, a growth inhibiting effect on *Trichomonas vaginalis in vitro* in concentrations of 0.004–0.006 mg/ml.

Borrelidin is a substance of considerable toxicity. The LD50 for mice was found to be 74.7 mg/kg and 39.0 mg/kg if given by the subcutaneous and intravenous route respectively. Other animals, *e.g.*, rats and rabbits, were considerably more sensitive to this material. Moreover, the new antibiotic is irritant to the tissues of animals and sometimes to the skin of humans.

Candidulin: an Antibiotic from Aspergillus candidus. P. G. Stansly and N. H. Ananenko. Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Conn.

A substance has been recovered from the fermentation liquor of a strain of *Aspergillus candidus* which has marked activity against acid-fast bacteria. The properties of the active principle distinguish it from known antibiotics. The name "candidulin" is proposed for it.

Moderately active fermentation liquor may be obtained in stationary culture on a neutral substrate consisting of glucose, sodium nitrate and potassium phosphate. The active principle may be removed from the metabolic liquor with chloroform and crystallized from n-hexane. The yield of purified material is about 5 mg. per liter. The substance is a colorless, neutral, non-aromatic compound, m.p. 88–89°, $[\alpha]_D^{24} = +15°$ (chloroform). Elemental analysis is consistent with an empirical formula of $C_{11}H_{15}NO_3$. Treatment with bromine in carbon tetrachloride afforded a biologically inactive crystalline product containing bromine, m.p. 143–147° (dec.).

In broth dilution tests, the antibiotic exhibits moderate activity against some Gram-positive and Gram-negative bacteria. It is particularly effective against the mycobacteria, inhibiting the growth of five strains, representing pathogenic and saprophytic species, in concentrations of approximately 1 μg. per ml. The substance, however, failed to influence the course of experimental mouse tuberculosis when administered subcutaneously in daily doses of 25 to 200 mg. per kg.

Inhibitory Action of Filtrates from Fungous Cultures on Three Plant Viruses.[1] W. C. Price, B. M. Gupta, and Sylvia Wolcyrz. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa.

Filtrates from three-weeks-old cultures of about 40 species of fungi grown on bacto-beef-cornmeal-dextrose broth were tested for ability to inhibit infection with Southern bean mosaic, tobacco mosaic, and tobacco ringspot viruses. Filtrates from *Trichothecium roseum, Neurospora sitophila,* and *N. crassa* were found to inhibit infection with all three viruses. The inhibitory action took place immediately whether the filtrate was mixed with the virus or applied to the host before or up to 30 minutes af-

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

ter inoculation. Virus preparations rendered inactive by addition of filtrate regained their activity when diluted. These results can be interpreted to mean that the filtrates either render the host insusceptible to the virus or enter into a reversible combination with the virus. Evidence favoring the former interpretation was obtained by study of the reduction in infectivity induced by addition of varying quantities of antiviral agent to a constant dilution of virus. The reduction in infectivity, as expressed in probits (or normal equivalent deviations), was found to be a linear function of the logarithm of dilution.

Marine Organisms as a Source of Physiologically Active Substances. Ivor Cornman. Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Every phylum of the animal kingdom has members which possess poisonous tissues or toxic secretions. Representative species have been tested according to the method outlined in an accompanying abstract.

The protozoan, *Gymnodinium brevis,* believed responsible for the "red tide" which periodically kills millions of fish and shellfish, produces a poison which checks the division of sea-urchin eggs.

Sponges crushed in sea water vary in their inhibitory potency. Of ten species tested, two exerted a slight effect only after several hours when diluted 1 : 100. Four species retarded the first cleavage at 1 : 100 and showed a delayed effect at 1 : 1,000. One allowed only 7 per cent of the eggs to divide when the sponge macerate was diluted 1 : 1,000 and retarded cleavage at 1 : 10,000. Another produced no measurable effect for several hours and then killed the larvae at all doses tested (1 : 100, 1 : 1,000, 1 : 10,000). These differences in dose-effect relationships reveal a qualitative as well as a quantitative difference in the active agents. The most toxic sponge species permitted only a few eggs to divide and visibly damaged the cytoplasm in a half hour when diluted 1 : 10,000. Even at 1 : 100,000 it killed the embryos but required several hours to take effect.

The spines of *Centrechinus* are poisonous enough to produce a painful swelling when they puncture the skin, and many species of fish cannot survive in tanks containing these sea urchins. A brei prepared from the spines proved to be only a weak inhibitor of cleavage.

Numerous mollusks release an ink when disturbed. A large sea slug, *Tethys,* ejects a raspberry-odored red fluid which blocks egg development at 1 : 1,000. *Thais patula* exudes a white garlic-odored fluid which turns blue. This blue solution retards cleavage when diluted 1 : 10 and is without influence at 1 : 100. Mantle fluid from *Thais deltoidea,* which has no ink, is harmless at 1 : 10.

Aureomycin and Chloramphenicol

The Assay of Aureomycin by Instrumental Methods. Homer Stone Kelsey and Leon Goldman. Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

Two methods based on a standard solution of aureomycin HCl are outlined for measuring the potency of aureomycin solutions.

A fluorometric method involves a five-min. boiling of 100–400 μg. of the antibiotic with an equimolecular mixture of Na_3PO_4 and Na_2HPO_4. On dilution, the resulting fluorescence is measured on a Pfaltz and Bauer fluorophotometer, using the same filter system as the Vitamin B_1 assay. A blank solution, made up to the same dilution, employs a PO_4 buffer to give a pH = 10, being read immediately without heating. The difference between readings represents the amount of Aureomycin present.

A colorimetric method uses a sample ranging from 100 to 1,400 μg. This sample is treated with twice its volume of conc. HCl for 10 min. when it is diluted for reading on a Lumetron Colorimeter (Model 402-E) using M440 filter. A blank solution is prepared using the same ingredients but adding the acid to the diluted sample. The galvanometer is set at 100 per cent transmittance with the blank solution after which the "treated solution" is read.

Both methods give a straight line standard curve on linear paper. Both give results comparing favorably with a microbiological assay method.

Studies on Assay Methods of Aureomycin in Body Fluids.[1] C. M. Whitlock, Jr., A. D. Hunt, Jr., and S. G. Tashman. Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania, Philadelphia, Pa.

The accuracy of the two basic methods of aureomycin assay which seemed most promising (namely, the serial dilution method of Dornbush and the turbidimetric method of Meads) was assessed by measuring known quantities of aureomycin in the presence of various human body fluids with both methods. Similar data have not been previously reported. For concentrations greater than 0.1 μg./cc., the serial dilution method was found to be accurate within ± 100 per cent in serum and urine, and to be inaccurate in spinal fluid (errors of 100 per cent or over being common). For similar concentrations, the turbidimetric method was found to be accurate within ± 50 per cent in serum, and ± 100 per cent in urine and spinal fluid.

In the above comparison, the technique used was essentially the same as that of the originators of the methods. When the turbidimetric method was modified by substituting a standard curve containing four points for the single standard used by Meads, the experimental error was reduced to within ± 25 per cent in serum, ± 35 per cent in spinal fluid, and ± 45 per cent in urine. This change also simplifies the method by eliminating most of the mathematical detail work. This laboratory now uses this modified turbidimetric method exclusively for the assay of aureomycin.

[1] These studies were conducted under contract with the Department of the Army, Chemical Corps, Camp Detrick, Md.

Investigation of plate methods of assay was terminated with inconclusive results when it was learned that this type of method measures aureomycin only in concentrations of about 1 μg./cc. or greater.

Using the turbidimetric assay method, it was determined that serum and acid urine specimens can be frozen and assayed in weekly lots without appreciable loss of aureomycin activity. Spinal fluid must be run on the day collected, however, if concentrations of less than 1 μg./cc. are to be accurately measured. When 1 μg./cc. of aureomycin in serum was incubated three hours at 37° C. before assay, there was less than 15 per cent loss of potency. This observation suggests that the degree of destruction of aureomycin under physiological conditions has been over-emphasized.

Aureomycin Studies. I. Effect of Aureomycin on Ten Strains of Virus in the Psittacosis-LGV Group. JOHN C. WAGNER. Biological Department, Chemical Corps, Camp Detrick, Frederick, Md.

A recent report (Cox *et al.*) on the efficacy of aureomycin on the 6BC strain of psittacosis virus made it appear desirable to obtain additional data on the action of this antibiotic on other virus strains within the psittacine group. This report presents the results of such a study in both eggs and mice on ten strains of virus in the psittacosis-lymphogranuloma venereum group.

Drug effect was evaluated in eggs by comparing the LD_{50} values of each virus strain titrated in untreated as compared to aureomycin treated embryos. In animal studies mice were inoculated intracerebrally with each virus strain and treated, beginning 48 hours later, by five daily subcutaneous inoculations of varying amounts of aureomycin.

In the egg experiments all the virus strains were extremely susceptible to aureomycin. In high dilution the virus was almost completely masked by the presence of drug as manifested by survival of eggs and low titer of virus on subinoculation. As the virus concentration increased, the masking effect could be demonstrated only by survival of treated embryos, but not by titer of recoverable virus within these embryos. All five pathogenic strains (Borg, S-F, Gleason, 6BC, P207) tested in mice were susceptible to the drug. Injections of 0.5 mg. or 0.1 mg. of aureomycin lengthened significantly the life span of treated groups of animals as compared to untreated controls. Living virus was recovered after 21 days from the brains of all treated groups of surviving mice.

The Action of Aureomycin on the Virus of Atypical Pneumonia in Chick Embryos and Cotton Rats. MONROE D. EATON. Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

The virus isolated from patients with atypical pneumonia as described in previous publications [1] is unrelated antigenically to Q fever or to agents of the psittacosis group, both of which are inhibited by aureomycin. Chick embryos inoculated by the amniotic route with the atypical pneumonia virus received two doses of aureomycin of 1 mg. each into the yolk sac two and 48 hours after the virus was given. The lungs, trachea and amniotic membrane of the treated embryos were pooled, made up in 20 per cent suspension, and inoculated intranasally into cotton rats to test for the presence of virus. Control embryos receiving virus and saline were similarly tested. The cotton rats were sacrificed ten days after inoculation. Of the 27 cotton rats receiving material from the treated eggs, one developed pulmonary lesions, while 20 of the 26 animals receiving control material developed areas of consolidation. When a single dose of 1 mg. of aureomycin was given two hours after the virus, treated eggs produced lesions in eight of 28 cotton rats, and controls in 17 of the 26 cotton rats, a less significant difference probably due to early decomposition of aureomycin in the chick embryo.

Direct treatment of cotton rats infected intranasally with chick embryo material and given daily intraperitoneal doses of 1 to 2 mg. of aureomycin starting 24 hours after inoculation of the virus also resulted in significant therapeutic effects on the pulmonary lesions. The incidence of pulmonary lesions in animals treated in this manner was as follows: 0/9 in those receiving 2 mg. daily for ten days, 1/22 in those receiving 1 mg., and 27/46 in the controls. Preliminary results indicate that the minimal therapeutic dose in cotton rats may be close to 0.5 mg. daily or approximately 10 mg./kg. body weight.

Clinical Evaluation of Aureomycin and Chloramphenicol in Pneumonia in Infants and Children. BENNETT OLSHAKER, SIDNEY ROSS, ADRIAN RECINOS, and ELLSWORTH TWIBLE. Children's Hospital, Washington, D. C.

Aureomycin

Forty cases of pneumonia in infants and children have been treated with aureomycin during the past three months at Children's Hospital. Of these, 32 were pyogenic pneumonias including pneumococcus, streptococcus and staphylococcus. The other eight cases appeared to satisfy the diagnostic criteria of atypical pneumonia.

In the pyogenic group the response both clinically and bacteriologically was striking. The temperature came down precipitously within 12 to 36 hours in almost every case and concomitantly a marked clinical improvement ensued. Roentgenologically, the resolution of the pneumonic process was rapid in the majority of cases; however, in about 20 per cent of the cases there was a lag in the resolution of the area of consolidation by x-ray approximating five to eight days. The drug was well tolerated orally with the exception of the occasional occurrence of nausea, vomiting and diarrhea. Ten of the cases in the infant age group received the drug intramuscularly and showed a prompt response in the majority of instances. However, it was our impression that the intramuscular mode of administration was less satisfactory therapeutically than the oral method of administration.

[1] Eaton, M. D., Meiklejohn, G., and Van Herick, W. J. Exp. Med., 1945, 82, 317–329. Am. J. Hygiene, 1947, 45, 82.

In the eight cases of atypical pneumonia the therapeutic response was similarly gratifying and seemed to follow the favorable course which Schoenbach and Bryer and Finland and his associates have described in their series of virus pneumonias in adults.

Chloramphenicol

Twenty-seven consecutive cases of bacterial pneumonia in infants and children were treated with chloramphenicol. The age range varied from five months to eight years. In 25 of the 27 cases, there was a prompt defervescence of temperature within 12 to 36 hours after initiation of the drug accompanied by marked clinical improvement. The white blood count dropped sharply and the areas of consolidation on x-ray showed resolution within three to six days. The results thus far would indicate that chloramphenicol has definite therapeutic value in bacterial pneumonia.

Some Observations on the Absorption and Excretion of Aureomycin and Chloramphenicol. SIDNEY ROSS, HAROLD BISCHOFF, WARREN PREISSER, and WILLIAM ORR. Children's Hospital, Washington, D. C.

A. Chloramphenicol

(1) Chloramphenicol was observed to pass through the placental barrier and was present in cord blood in a concentration of 50 to 75 per cent of that observed in the maternal blood.

(2) Chloramphenicol readily passed the blood-brain barrier and appeared in the spinal fluid in concentrations ranging from 30 to 50 per cent of that noted in the blood stream after oral administration.

(3) No enhancement in chloramphenicol blood levels was noted after concomitant oral administration of caronamide.

(4) Chloramphenicol was readily absorbed after rectal administration of 125 to 250 mgms. in infants and appeared in the blood in therapeutic concentrations.

B. Aureomycin

(1) Aureomycin was found in the pleural and peritoneal fluids of a nephrotic child after oral administration of 250 mgms. every four hours. The concentration of aureomycin in these fluids was approximately one-eighth of that noted in the blood.

(2) Aureomycin was absorbed into the blood stream after rectal administration of 250 mgms. in children.

Observations on Tuberculous Meningitis Treated with Oral Aureomycin.[1, 2] GEORGE T. HARRELL, MANSON MEADS,[3] ROBERT B. LAWSON, and THOMAS N. LIDE. Departments of Internal Medicine, Pediatrics and Pathology, Bowman Gray School of Medicine of Wake Forest College and the North Carolina Baptist Hospital, Winston-Salem, N. C.

In vitro observations showed that tubercle bacilli were susceptible to aureomycin. This antibiotic was found to penetrate into the spinal fluid after oral administration. These facts suggested possible application of the drug to therapy in tuberculous meningitis.

A four-year-old boy weighing 14 kilo. had fulminating symptoms of meningitis for three days. Acid-fast rods were found in large numbers on direct smear of spinal fluid. A critical study was done on the effect of very large doses of aureomycin (300–500 mg. per kilo., 4–8 grams daily) given only orally over 21 days. Almost daily blood and spinal fluid levels of aureomycin were obtained. Blood levels (2–15 μg./cc.) varied more widely than spinal fluid levels (1–3 μg./cc.) and both varied reciprocally with the number of stools (one to eight per day). Organisms disappeared on smear and the spinal fluid sugar rose from 10 to 40 mg.%. Clinical improvement was steady for over two weeks; a sudden worsening in the third week terminated fatally.

At autopsy caseous lesions were found in lymph nodes and spleen with no evidence of healing. The drug apparently did not reach intracellular organisms deep in tubercles. Serial cultures were planted directly and the susceptibility of the organisms to aureomycin was determined after recovery from guinea pigs inoculated with spinal fluid and tissue.

No toxic effects were noted pathologically from this huge dose—the largest yet recorded—administered for a relatively long time. The negative spinal fluid cultures without intrathecal therapy may justify further study of aureomycin in combination with other forms of therapy in this disease.

A Comparative Study on the Effects of Aureomycin and Penicillin on the Pharyngeal Flora of Normal Human Beings.[1, 2] MANSON MEADS,[3] WALLACE P. ROWE, and NANCY M. HASLAM. Department of Internal Medicine, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

The effects of aureomycin and penicillin on the aerobic bacterial flora of the pharynx were compared in normal human subjects. Repeated semiquantitative throat cultures were taken from the same individual before, during, and after the successive oral administration of the two antibiotics in different doses. By utilizing each subject as his own control, host factors that may influence chemo-

[1] This study was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] The aureomycin used in this study was furnished by Lederle Laboratories.

[3] Markle Scholar in Medicine.

[1] This investigation was supported (in part) by a research grant from the Bacteriology Study Section, Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] The aureomycin and penicillin used in this study were furnished by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., through the courtesy of Dr. Stanton M. Hardy.

[3] Markle Scholar in Medicine.

therapeutic response were minimized. Repeated tests were made of the *in vitro* susceptibility of the bacteria recovered throughout the period of observation. The *alpha hemolytic* streptococci proved to be a good index for the comparison of certain aspects of the antibacterial activity of the two drugs because of (1) their susceptibility to aureomycin and penicillin, (2) their constant appearance in normal pharyngeal cultures, and (3) their consistent reappearance after effective therapy had been stopped.

With the exception of the genus *Hemophilus,* aureomycin and penicillin demonstrated a similar spectrum of antibacterial activity. By weight, penicillin was approximately five times more effective than aureomycin against alpha streptococci in the human pharynx; penicillin was 12 times more active against these organisms *in vitro.* In most instances, a drug-specific decrease in the mean *in vitro* susceptibility of alpha streptococci and *Neisseria flava* paralleled a failure to clear these organisms from the pharynx. The mean increase in resistance to penicillin was maintained throughout a three-week period after this drug had been discontinued. The degree of aureomycin-fastness that followed treatment with this agent slowly decreased *in vivo,* although repeated transfer of resistant organisms in media free of antibiotic failed to effect their degree of susceptibility. The apparently stable property, fastness to penicillin, was used as a label to study the dynamics of aureomycin-fastness in pharyngeal bacteria.

This method of antibiotic comparison may serve as a valuable tool to supplement laboratory studies on a new chemotherapeutic agent before it is used in clinical disease.

Isolation and Chemistry of Chloramphenicol (Chloromycetin[1]*).* QUENTIN R. BARTZ. Research Laboratories, Parke, Davis and Company, Detroit, Mich.

The activity of chloramphenicol, a new crystalline antibiotic obtained from filtrates of submerged aerated cultures of *Streptomyces venezuelae,* was followed by means of a turbidimetric method employing *Shigella paradysenteriae* (Sonne) as the test organism. It was stable at 25° in culture filtrates over the pH range of 0.40–9.56 for 24 hours but was rapidly destroyed at pH 10.82. The distribution ratio at 25° of chloramphenicol between organic solvent and either acidified or weakly alkaline aqueous solution was found to be three or more for cyclohexanone, butanol-1, ethyl acetate, methyl isobutyl ketone, isopropyl acetate, amyl acetate, methyl amyl acetate, nitrobenzene, nitromethane and diethyl ether; approximately one for ethylene dichloride, and less than one for chloroform, carbon tetrachloride, trichloroethylene, benzene, dichloroethyl ether, and petroleum ether. Chloramphenicol is readily absorbed by Nuchar C190N from aqueous solution at either an acid or neutral pH.

Two general procedures for the isolation of chloramphenicol in crystalline form from crude culture filtrates involve (1) extraction with water-immiscible organic solvents or (2) adsorption on carbon and elution with organic solvents.

Chloramphenicol is an optically active, nonionic chlorine-containing amide, melting at 149.7–150.7°, the molecular formula of which is $C_{11}H_{12}Cl_2N_2O_5$. The assigned structure has been confirmed by an unequivocal synthesis (Controulis, Rebstock and Crooks).

[1] The name Chloromycetin has been adopted by Parke, Davis & Company as a trademark for this antibiotic.

Chloramphenicol (Chloromycetin[1]*) Assay and Susceptibility Test Methods.* D. A. JOSLYN, J. EHRLICH, and J. L. SCHWAB.[2] Research Laboratories, Parke, Davis & Company, Detroit, Mich.

The chloramphenicol content of aqueous solutions and body fluids has been determined by three microbiological methods. *Turbidimetric Method:* Samples are diluted to an estimated concentration of 0.2 microgram chloramphenicol per milliliter in brain heart infusion (Difco) containing a standardized inoculum of *Shigella sonnei;* after *ca.* four hours' incubation at 37° C., potency is calculated by comparing turbidity of the sample tubes with that of control tubes containing 0.1 to 0.5 μg. of a standard chloramphenicol per milliliter. *Overnight Broth Method:* Samples are diluted serially as required in brain heart infusion containing a standardized inoculum of *S. sonnei;* after *ca.* 18 hours' incubation at 37° C., the concentration of chloramphenicol in the sample is estimated by comparing the end point (complete inhibition) of the sample with that of a standard. *Agar Diffusion Method:* Uniform volumes of appropriately diluted samples and standard are added to one-half inch diameter filter paper disks placed on agar plates seeded with *Bacillus subtilis* or other susceptible test organism; potency is estimated by comparing the resulting inhibition zone diameters with aid of a standard curve.

The susceptibility of cultures, particularly those isolated from candidates for chloramphenicol therapy, has been tested by two microbiological methods. *Agar Dilution Method:* Tryptose agar dilutions of chloramphenicol are streaked with clinical material; after suitable incubation, presence or absence of growth in the several plates indicates the degree of susceptibility. *Broth Dilution Method:* Tryptose broth dilutions of chloramphenicol are inoculated with a standardized suspension of the cultures on test; after suitable incubation, the degree of susceptibility is indicated by complete inhibition of growth.

A Colorimetric Method for the Determination of Chloramphenicol (Chloromycetin[1]*); Application to Biochemical Studies.* ANTHONY J. GLAZKO, WESLEY A. DILL, and LORETTA M. WOLF. Research Laboratories of Parke, Davis and Company, Detroit, Mich.

The colorimetric method used for the determination of Chloromycetin and related aromatic nitro compounds is based on reduction with metallic zinc or titanous chloride,

[1] Chloromycetin is the Parke, Davis and Company trade name for chloramphenicol.

[2] With technical assistance of M. C. Galbraith, D. Fox, M. L. Davitt, M. F. Sheehan and F. E. Guest.

followed by diazotization and coupling of the resulting aryl amine with the Bratton-Marshall reagent. The active antibiotic can be separated from its metabolic products by extraction with ethyl acetate and determined separately. The titanous procedure has also been used for the development of color on paper strips after chromatographic separation of various Chloromycetin derivatives.

Blood level studies on man and lower animals indicate rapid absorption and excretion of this antibiotic. Human subjects given Chloromycetin by mouth were found to excrete aromatic nitro compounds which accounted for more than 90 per cent of the administered dose in 24-hour urine specimens. Less than 10 per cent of this was found to be active Chloromycetin which was isolated from urine and identified.

The inactive metabolic products were chromatographed on paper strips, and one component was shown to have the same R_f value as a known hydrolysis product of Chloromycetin. The other inactive derivative, representing the major excretory product, was isolated by countercurrent extraction and tentatively identified as the mono-glucuronide. This metabolic product was readily hydrolyzed with β-glucuronidase, yielding active Chloromycetin.

No increase was observed in the urinary excretion of aryl amines in man following the administration of Chloromycetin, but lower animals such as the mouse, rat, and guinea pig excreted aryl amines in addition to inactive nitro compounds. *In vitro* studies with tissues of the rat and guinea pig demonstrated the presence of enzyme systems which converted Chloromycetin to inactive nitro compounds and also reduced the nitro groups to produce aryl amines.

Chloromycetin in Salmonellosis. S. Ross, F. G. Burke, E. C. Rice, and J. A. Washington. Children's Hospital, Washington, D. C.

Five cases of salmonellosis have been treated or are being treated with Chloromycetin at Children's Hospital. Two of these five cases are carriers who have failed to respond to streptomycin, aureomycin, or aerosporin during the past four months. The efficacy of Chloromycetin in these cases is too early to evaluate. The stool cultures are now negative for the first time in four months; however, insufficient time has elapsed for an adequate follow-up. The dose used was 125 mgm. every four hours orally for 10 to 14 days.

A third case had a positive blood culture for salmonella on admission. After administration of the drug, subsequent blood cultures became sterile and a positive stool culture was not obtained at any time. The child made an uneventful recovery. A fourth case currently under treatment has shown a persistence of positive stool cultures during the first four days after initiation of the drug.

The fifth case was a critically ill child with a positive blood culture. Chloromycetin was started shortly after the positive blood culture was obtained; however, the child died within 12 hours. The duration of therapy was too short to permit any evaluation of the efficacy of the drug in this case.

It would seem thus far that Chloromycetin might be more promising in salmonellosis than any antibiotic we have used; however, a larger number of cases and a longer follow-up would be mandatory before any categorical statement could be made.

Chloromycetin[1] *in Therapy of Bacillary Urinary Infection.* George E. Chittenden, Elwood A. Sharp, A. J. Glazko, and A. S. Schlingman. Research Laboratories, Parke, Davis and Company, Detroit, Mich.

When the clinical investigation of the antibiotic effect of Chloromycetin in bacillary urinary tract infections was begun, only fragmentary data on blood levels, urine concentrations and excretion rates were available. As a result, numerous dosage patterns were studied. Some patients were given large (3.0 Gm.) single doses and were followed by frequent urinary cultures and blood levels. Others were given 2.0 Gm. single doses followed by 0.5 to 1.0 Gm. at four to eight hour intervals.

As a result of a study of approximately 50 patients, the following facts have been determined:

(1) Chloromycetin appears to be a safe drug, especially when given orally in a wide range of dosage patterns. Thus, in mild to moderate bacillary infections, 0.5 Gm. every six to eight hours around the clock will maintain a satisfactory therapeutic blood level. In severe cases, a large initial dose (1.0 to 3.0 Gm.) followed by maintenance doses of 0.5 Gm. every six to eight hours will be adequate.
(2) Therapy may be continued for a long period of time with no adverse pathological reactions. In one case, approximately 54.0 Gm. were given continuously without toxic or allergic reactions.
(3) Since our studies have indicated the frequent presence of a multiplicity of organisms (Gram-positive and Gram-negative), treatment of urinary infections with sulfadiazine or penicillin in addition to Chloromycetin may be indicated.

Aureomycin and Chloramphenicol: Use in Typhus, Typhoid and Brucellosis. Vernon Knight, Walsh McDermott, and Francisco Ruiz-Sanchez. Department of Medicine of the New York Hospital-Cornell University Medical College, New York, N. Y., and the Institute for the Experimental Study of Infections of the University of Guadalajara, Mexico.

Twenty-eight patients with typhus fever, presumably of murine variety, have been treated with aureomycin. The majority of a group of 23, treated by oral administration, received 100 to 200 mg. per Kg. per day for periods of one to two days with dramatic improvement and prompt clinical remission.

Other patients were treated with lower oral doses and by the intravenous route. These studies indicated that 50 mg. per Kg. per day orally for 36 to 48 hours was the lowest limit of effective dosage and that a dose of 25 mg.

[1] Trade-Mark Parke, Davis and Company for chloramphenicol.

per Kg. per day for 36 hours was sub-effective. Sub-effective and effective doses of 400 mg. and 1,000 mg. intravenously respectively in a 48 hour period of time were observed.

It was concluded that aureomycin constitutes highly effective therapy for typhus fever and that an approximate oral-intravenous ratio of effectiveness of one to five exists in this disease.

Twenty-three patients clinically ill with brucellosis, of whom nine had positive blood cultures for *B. melitensis,* were treated with aureomycin and chloramphenicol. The treated groups were almost equally divided and received similar doses of these drugs for periods averaging about eight days in doses ranging from 50 to 100 mg. per Kg. per day. Prompt clinical remission occurred in both groups after therapy. No significant differences were observed in the degree and duration of fever after therapy, although the clinical impression was gained that slightly more chloramphenicol than aureomycin was necessary to produce comparable results in the treatment of this infection. Frequent relapses make necessary further study of dosage regimens and evaluation of therapy in terms of permanent remission.

Forty-six patients with typhoid fever, of whom 35 were bacteremic, were treated with chloramphenicol or aureomycin. Dosages of aureomycin ranged from 50 to 500 mg. per Kg. per day for approximate eight day intervals. Chloramphenicol was given in most cases in doses of 100 mg. per Kg. per day for a similar period of time.

Clinical observations revealed that chloramphenicol was far superior to aureomycin in the treatment of typhoid fever, and this was in conformity with the previously reported good results with chloramphenicol. A definite, but slight effect was observable, however, in the aureomycin-treated patients.

Polypeptide Antibiotics

Chemical Studies of Circulin.[1] D. H. PETERSON and L. M. REINEKE. Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

Circulin, an antibiotic more active against Gram-negative than Gram-positive organisms, was isolated and purified from the fermentation liquors of a non-hemolytic strain of *B. circulans* by Peterson and Colingsworth, working in cooperation with Murray, Tetrault, Kaufmann and Koffler.

Circulin, like the polymyxins, is a basic polypeptide, but differs from them in amino acid composition and is inactivated by lipase. The active principle was considered pure when repeated carbon chromatography and regeneration from its salts failed to raise the activity or alter its chemical composition. Circulin readily forms salts such as the hydrochloride, picrate, reineckate and helianthate. The antibiotic is insoluble in the water immiscible solvents but is very soluble in water and less soluble in the lower alcohols. Circulin gives a negative ninhydrin-carbon dioxide reaction. Nevertheless, it contains free amino groups as exhibited by the Van Slyke and formalin reactions as well as by its reaction with 2,4-dinitrofluorobenzene.

An optically active isomer of pelargonic acid was isolated from the acid hydrolysate of circulin. Paper strip chromatography, microbiological and isolation studies of this hydrolysate demonstrated the presence of L-α,γ-diaminobutyric acid, L-threonine and D-leucine. From 300 mg. of acid hydrolysate it was possible to separate the amino acids by powdered cellulose partition chromatography in sufficient quantities to identify the constituents by various chemical means.

Paper strip chromatography reveals that the only free basic amino groups of circulin are due to the γ-amino group of α,γ-diaminobutyric acid.

Studies on Circulin. P. A. TETRAULT, H. KOFFLER, O. W. KAUFMANN, and L. Y. QUINN. Laboratories of Bacteriology, Department of Biological Sciences, Purdue University, Lafayette, Ind.

The discovery of an antibiotic which is very active against Gram-negative bacteria and is related to the polymyxins was recently announced by Murray and Tetrault (Proc. Soc. Am. Bact., 1948, 1, 20). This antibiotic is produced by *Bacillus circulans* Q-19 and was therefore named *circulin* in a paper in press which also summarizes information on its assay, isolation, and general properties (Murray, Kaufmann, Tetrault, Koffler, Peterson, and Colingsworth, J. Bact., 57, in press). This antibiotic is not identical with the highly toxic product of *Bacillus krzemieniewski* M-14 which is being studied at the Venereal Disease Research Laboratory and which until recently was also named circulin (McLeod, J. Bact., 1948, 56, 749–754.

Yields which are approximately equivalent to from 50 to 100 mg. of pure circulin sulfate per 100 ml. of medium can be obtained during the submerged growth of *Bacillus circulans* Q-19 in a medium which contains the following: 2 per cent yeast extract, 2 per cent dextrin, 1 per cent $(NH_4)_2SO_4$, 0.4 per cent KCl, 0.02 per cent KH_2PO_4, and 1.6 per cent $CaCO_3$. The following chemical changes occurred during a representative fermentation: Bacterial growth proceeded fairly rapidly after inoculation, especially so after 12 hours. Maximum cellular development was reached approximately after 36 hours. Highest circulin yields were obtained 12 hours later. At that time the cells had begun to autolyze. Organic nitrogen compounds appeared to be used in preference to ammonia nitrogen. After 36 hours almost all of the organic nitrogen had been utilized while the concentration of ammonia nitrogen remained relatively unchanged. The utilization of dextrin paralleled bacterial growth. After 36 hours, when a maximum population level had been reached, dextrin was no longer used, although approximately one-fourth of the original amount still remained. While utilizing dextrin the culture produced lactic acid which it then kept on using until it reached maximum growth.

[1] In a recent note to the J. Bact. (In Press) the name Circulin was withdrawn in favor of Mucoidin for an antibiotic described by Charlotte McLeod, J. Bact., 1948, 56, 749.

The pH values reflected these chemical changes. A drop in pH from 7.8 to 6.4 accompanied the production of lactic acid; when the lactic acid was utilized and nitrogen compounds were released, the pH rose from 6.4 to 7.4, and then more gently to 7.8, a pH value reached after 73 hours. Preliminary experiments indicated that circulin protects white mice against infections caused by *Salmonella typhosa, Klebsiella pneumoniae,* and *Vibrio cholerae.* The amounts of circulin necessary to accomplish this were from one-seventh to one-fourth as large as the amounts lethal to half of the mice tested.

A Consideration of the Toxicity of Bacitracin Following Systemic Administration. FRANK LAMONT MELENEY and BALBINA JOHNSON. Columbia University, College of Physicians and Surgeons, New York, N. Y.

There is no evidence of toxicity or allergy from the local injection of bacitracin in the treatment of localized surgical infections from the surface application of bacitracin, or from mouth administration of single doses as high as 250,000 units. Nevertheless, evidences of kidney irritation have appeared following the intramuscular injection of doses ranging from 10,000 to 100,000 units administered every four to six hours. These toxic effects have varied strikingly with the different manufactured lots and with different manufacturing methods. This was not fully recognized until June, 1948.

Since July, precautions have been taken to use only those lots which have demonstrated an LD 50 of 300 or more for a 20-gram mouse. In the last eight months, 60 or more cases have been treated successfully with these lots without any evidence of serious kidney damage. This report covers more than 200 cases which clearly reveal not only the variations in toxicity but the safety and effectiveness of lots which have demonstrated, by the FDA tests, an LD 50 of 500 for 20-gram mice. Sixty per cent of these cases have been treated with bacitracin because they failed to respond to other methods of antibacterial therapy and more than half of these have then responded to bacitracin.

Recent experience indicates that there is an increasing number of bacterial infections which do not respond to penicillin and the causative organisms are frequently found to be resistant to penicillin but susceptible to bacitracin. These facts clearly indicate the need for an antibiotic with the attributes of bacitracin.

Bacitracin Therapy of Experimental Staphylococcal Meningitis in the Dog. PAUL TENG and FRANK LAMONT MELENEY. Columbia University, College of Physicians and Surgeons, New York, N. Y.

Early experiments in normal animals demonstrated that bacitracin was held back by the blood-brain barrier and reached the cerebrospinal fluid in only about 10 per cent of the concentration attained in the blood, following intravenous or intramuscular injection. However, this concentration was considerably increased in the presence of local inflammation.

Following the intracisternal injection of 3 cc. of saline containing 10,000 units of bacitracin in normal dogs, there was some initial stiffness of the neck and pleocytosis; but the animals appeared normal as soon as they recovered from the anesthetic and there was no evidence of injury to the brain or cord.

In a series of 16 dogs in which meningitis was produced by the intracisternal injection of 500 million staphylococci, one-half of the animals were retained as controls while the other half were treated with the intracisternal injection of bacitracin at varying intervals following the inoculation of the organisms. In all of the animals, symptoms of meningitis rapidly developed and in the controls it was invariably fatal in from four and one-half to 37 hours, but the treated animals survived if treatment was begun within three hours following the inoculation of organisms. The intracisternal treatments varied from one to three in number, ranging from 4,000 to 10,000 units contained in 3 cc. of saline. Intracisternal treatment was supplemented by intramuscular injections of bacitracin varying from one to eight in number, ranging from 10,000 to 50,000 units. Intramuscular injections alone, containing 25,000 units every six hours, in four animals failed to save any of them.

These experiments indicate the trial of bacitracin by the intrathecal route in patients with meningitis due to organisms susceptible to bacitracin.

Parenteral Bacitracin in Surgical Infections. ALFRED B. LONGACRE, ROBERT M. WATERS, and FLORENCE EVANS. Department of Surgery, Louisiana State University, School of Medicine, New Orleans, La.

Bacitracin is an antibiotic derived from the Tracey strain of *Bacillus subtilis.* It exerts its greatest antibiotic action against species and strains of Gram-positive cocci. However, it is also an effective antibiotic against the clostridia organisms and also many strains of spirochetes including *T. pallidum.* As with the other antibiotics its full range of antibiotic activity will only be determined by further investigation of its inhibiting action on various strains of bacteria.

In this study of 50 cases of infection, bacitracin was an effective therapeutic agent in 33 or 65 per cent of the cases. It was administered parenterally in varying doses, ranging from 2,000 to 52,000 units every six hours. Some of the cases also received bacitracin locally in addition to the parenteral dose. The types of infection studied include those of cellulitis, deep tissue abscesses, infected wounds, furuncles and carbuncles, human bite and other miscellaneous infections. All bacteria recovered from the infections were tested for bacitracin sensitivity. In 25 instances the flora contained at least one bacitracin-sensitive organism and in this group 19 or 76 per cent responded favorably, whereas in the group of eight cases with only resistant strains, only three or 37.5 per cent had a favorable response. There were 16 cases, mostly those of cellulitis, from which no cultures were obtainable. In this last group 12 or 75 per cent had an excellent or good response.

The usefulness of parenteral bacitracin is at present restricted because of its toxic reactions. In this series, 16 cases demonstrated changes indicative of a possible lower

nephron syndrome. However, in only two instances were these changes of sufficient magnitude to be considered serious, and in no instance did a reaction persist after cessation of administration of the antibiotic. The exact factor responsible for these reactions is not known but the accumulated evidence suggests that it probably is not in the bacitracin itself but is a part of the mixture. The incidence of reactions varied with the different lots of bacitracin and did not appear to vary with the size of the dose or type of infection.

Even though 50 cases is a very small number of cases, the clinical response in those responding favorably indicates that bacitracin is an antibiotic of definite therapeutic value in cases which are caused by bacteria sensitive to it.

The Use of Aerosporin in Specific and Non-specific Enteritis in Infants and Children. FREDERIC G. BURKE, SIDNEY ROSS, E. CLARENCE RICE, and JOHN A. WASHINGTON. Children's Hospital, Washington, D. C.

Forty cases of enteritis were treated with aerosporin. These included 18 cases of non-specific enteritis, 16 cases of shigella, 40 cases of salmonella enteritis and two cases of typhoid fever. The dose employed was 2 to 3 mgms. per kg. of body weight every four hours by mouth for seven to 15 days. The aerosporin sensitivity ranged from 0.02 to 0.08 μg. per ml. Within 48 to 72 hours there was a marked diminution of the stool bacterial flora but no particular benefit was noted throughout the course of the non-specific diarrhea. In 14 of the 16 shigella cases a cure was noted and in two no significant effect was observed.

The value of aerosporin in salmonella was equivocal and the drug was given intramuscularly and by mouth. Toxic manifestations intervened in two of the cases and precluded adequate evaluation. There was no striking effect of the drug in the two cases of typhoid fever.

Following intramuscular injection toxic reactions included local pain, fever, malaise, and leukocytosis. Nephrotoxic reactions were noted in cases given the drug by mouth and intramuscularly and consisted of azotemia, albuminuria, white cells and casts in the urinary sediment. The high incidence of toxic manifestations of the drug would preclude its widespread usefulness.