Erythromycin

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ANTIBIOTICS MONOGRAPHS

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FOREWORD

The steady scientific progress in the field of antibiotics has had a powerful impact not only on clinical medicine but also on the amount of medical literature published. In the last decade the literature on antibiotics has attained such a tremendous volume that today it is difficult for the physician to keep up with the large number of publications that are made available to him.

The role of historical catalyst played by antibiotics in the evolution of modern medicine and the rich harvest of antibiotic literature have been determining factors in making the clinical and scientific monograph—that most useful literary form between an article and a book—one of today's most useful working tools for research men and medical practitioners. For a monograph permits the presentation of the multiple facets of a medical or laboratory problem as seen through the prism of the experience of a single author, who adds to his scientific wisdom that of many other workers in the same field.

This monograph is the first of a series which will bring to the general practitioner, the laboratory worker, the research man, and the medical specialist a concise and accurate picture of each of the principle antibiotics and their application in clinical medicine as well as in other allied fields of endeavor wherein antibiotics continually extend their spectrum of usefulness. Each one of these monographs will give the general practitioner the essence of current knowledge concerning the clinical use of a particular antibiotic as evaluated by an outstanding authority on the subject. It will also give the research worker the information he requires to develop his studies and will include, in addition, a selective and useful bibliography.

The first of these monographs is dedicated to erythromycin, one of the latest antibiotics. It is of singular importance because of its effectiveness against the staphylococci that makes erythromycin an important chemotherapeutic weapon in our modern arsenal against infection. We were fortunate indeed to prevail upon Dr. Wallace E. Herrell, one of the outstanding authors in the field of chemotherapy, to prepare this monograph. Doctor Herrell, a pioneer in the antibiotic field, and his colleagues at the Mayo Clinic have published innumerable basic articles on these drugs and their clinical usefulness. Doctor Herrell's interest has continued unabated in his present position in the Division of Medicine, Lexington Clinic, Lexington, Kentucky.

It is of historical interest to note that the earliest publication to establish erythromycin as an important chemotherapeutic agent was that of Herrell and his colleagues at the Mayo Clinic. The clinical observations of this group thoroughly established the pattern of therapeutic usefulness of erythromycin.

In this monograph Dr. Herrell presents in a concise but complete manner, in that terse and neat style that is becoming a characteristic of the modern man of science, the history of the development of this antibiotic, its antimicrobial activity, its pharmacology, and its clinical effectiveness in a wide variety of infections. He has drawn freely not only from his own clinical experiences but also from the most important and recent studies on the clinical efficacy of this useful chemotherapeutic agent.

The last chapter in the history of an antibiotic cannot yet be written, fortunately, and we hope it never will be. Erythromycin continues to rise in its therapeutic orbit as clinical investigation finds new indications for this drug. In this monograph Dr. Herrell has presented the current clinical scope of an important drug which has a recognized place in the field of antibiotic medicine.

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Chapter I

History and Production of Erythromycin

The isolation of erythromycin was first reported by McGuire and his colleagues[1] of the Research Laboratories of Eli Lilly and Company in June of 1952. The isolation of erythromycin resulted from the extensive screening program that has been in progress in the United States for several years. The particular soil sample yielding the organism that elaborated this antibiotic was collected in the Philippine Archipelago. The morphologic studies and the cultural characteristics of this organism indicated that it belonged to the genus *Streptomyces,* and it was further identified as a strain of *Streptomyces erythreus,* which had been described previously by Waksman and Henrici. When the organism was cultivated on a modified tryptose-phosphate agar, test plates showed activity against gram-positive, as well as some gram-negative, microbes. Likewise, the broth obtained when the organism was grown on a soybean meal-glucose medium showed activity against the gram-positive bacteria, as well as mycobacteria. According to McGuire and his colleagues,[1] the material possessed some activity against typhus rickettsiae and certain large viruses.

When the filtrate was made basic and extracted with benzene, ethyl acetate, carbon tetrachloride, etc., a crystalline antibiotic was isolated. Since that time the substance has been known as erythromycin but was given the trade name "Ilotycin" by the investigators at Eli Lilly and Company.

Physical and Chemical Properties

Erythromycin is a basic compound that is soluble to the extent of approximately 2 mg./ml. in water. It has been found to be extremely soluble in alcohols, acetone, chloroform, acetonitrile, and ethyl acetate and moderately soluble in ether, ethylene dichloride, and amyl acetate. It readily forms salts with acids. The preliminary estimated molecular weight of erythromycin from the titration data reported by McGuire and his col-

leagues[1] was approximately 725. Clark[2] suggested a probable molecular formula of $C_{39}H_{73}NO_{18}$ and a molecular weight of 766. Flynn,[3] in a later report, has shown the molecular weight to be 733.9 to 735.9 and the empirical formula to be $C_{37}H_{67\text{-}69}NO_{13}$.

According to Hasbrouck and Garven,[4] hydrolysis of erythromycin with dilute hydrochloric acid yields two products, a neutral oil, $C_8H_{16}O_4$, and a crystalline base, $C_{29}H_{49}NO_8$. The neutral oil consists of the cyclic hemiacetal of a hydroxyaldehyde containing two hydroxyl, one methoxyl, and two C-methyl groups. The basic C_{29} fragment contains two active hydrogen atoms and five C-methyl groups. It appeared to these investigators that the various fragments obtained from the acid hydrolysis of erythromycin are originally joined by acetal linkages.

As was pointed out earlier, erythromycin is a basic substance. It goes slowly into solution in water. Solutions are relatively stable and will maintain their full activity for several weeks in the frozen state. In physiologic saline solution erythromycin will maintain its full activity after standing for 24 to 48 hours at room temperature. After three days at room temperature, there is a slight loss of activity. Like streptomycin, erythromycin is more active at *p*H 8 than at *p*H 7.

Chapter II

Antimicrobial Activity of Erythromycin

Contrary to early suggestions that erythromycin was another so-called broad-spectrum antibiotic, it is a highly selective antimicrobial agent. From the early reports, it appears that erythromycin is similar in its action to penicillin. However, as published data have accumulated, it has become evident that the spectrum of erythromycin not only includes those organisms susceptible to penicillin but also has some action against *Hemophilus influenzae* and *H. pertussis* organisms, *Brucella melitensis* and *B. suis*, and the large viruses and has a suppressive action of the amoeba.

In Vitro and in Vivo Activity

It was evident from the reports by McGuire and co-workers[1] and Heilman and his colleagues[5] that the in vitro activity of erythromycin indicated that it could be expected to be a valuable therapeutic agent in the control of certain experimental and clinical infections. All strains of *Diplococcus pneumoniae* tested were quite sensitive to the action of erythromycin. All strains of *Streptococcus pyogenes* belonging to groups A, B, C, D, and E were fairly sensitive. *Streptococcus mitis* was quite sensitive. The average concentration of erythromycin required to inhibit 30 strains of *Streptococcus faecalis* was 0.6 μg./ml. *Micrococcus pyogenes* (30 strains) was inhibited in a range of from 0.2 to 2.0 μg./ml. Corynebacteria, including *C. diphtheriae, C. pyogenes, C. renale,* and *C. pseudotuberculosis,* were among the most sensitive organisms tested. *Listeria monocytogenes, Erysipelothrix rhusiopathiae, Bacillus anthracis,* and all strains of Clostridia, including *Cl. perfringens, Cl. septicum, Cl. tetani, Cl. histolyticum, Cl. novyi,* and *Cl. feseri,* were quite sensitive to the action of erythromycin in vitro. In general, the gram-positive bacteria were more sensitive than the gram-negative. The fungi tested by Heilman et al[5] were resistant. Of the gram-negative organisms, the enteric bacteria were highly resistant, but *H. pertussis* was quite sensitive. Four strains of *H. influenzae* were con-

sidered resistant to erythromycin. Welch[6] reported on 21 strains of *H. influenzae* and found the sensitivity to erythromycin ranged from 0.625 to 3.12 with a median of 1.56 μg./ml. *Neisseria gonorrhoeae* and *Neisseria intracellularis*, organisms of the genus *Brucella*, and *Actinomyces israeli* all were found to be moderately sensitive. Gorzynski and Neter[7] reported that erythromycin possessed considerable activity against *Pasteurella multocida*.

In a study of the in vitro susceptibility of *M. pyogenes* and enterococci to erythromycin, carbomycin, and a number of other antibiotics, including various combinations, Eisenberg and his colleagues[8] found that more than 90 per cent of the organisms tested were susceptible to erythromycin. In this study it appears that by comparison only 58 per cent of the strains tested were penicillin sensitive. Knight and Holzer[9] later reported a study on the antibiotic sensitivity of 516 strains of *M. pyogenes* cultured from staphylococcal carriers in 1953 and 1954. Sixty-five per cent were resistant to chlortetracycline, oxytetracycline, streptomycin, and penicillin. Most of the organisms were of the phage group III patterns. Nearly all strains were found to be highly susceptible to erythromycin. In another study on the general subject of the changing pattern of resistance of *M. pyogenes* to antibiotics, Kirby and Ahern[10] found only two strains that were resistant to erythromycin, and these occurred in patients who had received prolonged therapy with that antibiotic.

Based on the information available at the time of this writing, organisms that are susceptible or insusceptible to the action of erythromycin are listed in table I.

In vivo studies reported by Heilman and his colleagues[5] indicated that solutions of 1 mg. of erythromycin per ml. could be injected subcutaneously or intraperitoneally into animals with little evidence of irritation. Likewise, mice ate a diet of ground Purina laboratory chow containing 0.5 per cent of the antibiotic over a period of two weeks and gained weight. Mice fed such a diet for two days remained well when a number of lethal doses of *D. pneumoniae* were given intraperitoneally. Mice that received erythromycin by the intraperitoneal route were protected against 1000 lethal doses of the virus of psittacosis, which had been inoculated intraperitoneally. Guinea pigs were protected against experimental infections with *C. diphtheriae* when given erythromycin in adequate doses. That erythromycin was comparable to other available antibiotics in the control of experimental infections owing to virulent streptococci, pneumococci, and *H. pertussis* was evident from the report of Powell and his associates.[11] McCowen and co-workers[12] reported that erythromycin was effective

against experimental infections due to *Borrelia novyi* in mice and also had a protective effect against toxoplasma in mice. They found further that this antibiotic prevented death when given orally to mice infected with *Trypanosoma equiperdum.*

Thus, experimental studies with erythromycin indicated that trial of the antibiotic was warranted in the treatment of a number of clinical infections, including those caused by most of the commonly encountered pathogenic cocci, to organisms of the genera *Corynebacterium, Neisseria,* and *Clostridium* and to the organisms, *H. pertussis, E. rhusiopathiae, B. anthracis,* and *A. israeli.* On the other hand, from preliminary in vitro and in vivo studies it did not appear likely that erythromycin would be highly effective in the treatment of commonly encountered infections owing to pathogenic fungi, gram-negative enteric bacteria, *Aerobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris,* or organisms of the genus *Brucella.* Furthermore, experimental studies did not suggest that this antibiotic would be of value in the treatment of rabies, poliomyelitis, or influenza.

Heilman and his colleagues[5] reported that there was little or no evidence of cross resistance between erythromycin and other antibiotics commonly

TABLE I *Susceptibility of Microorganisms to Erythromycin*

Susceptible	Insusceptible
Diplococcus pneumoniae	*Nocardia asteroides*
Streptococcus pyogenes	*Mycobacterium tuberculosis*
Streptococcus mitis	*Candida albicans (Monilia)*
Streptococcus faecalis	*Blastomyces dermatitidis*
Micrococcus pyogenes	*Salmonella typhosa*
Corynebacterium diphtheriae	*Salmonella paratyphi* B
Corynebacterium pyogenes	*Escherichia coli*
Corynebacterium renale	*Aerobacter aerogenes*
Corynebacterium pseudotuberculosis	*Klebsiella pneumoniae*
Erysipelothrix rhusiopathiae	*Pseudomonas aeruginosa*
Bacillus anthracis	*Proteus vulgaris*
Actinomyces israeli	*Brucella abortus*
Listeria monocytogenes	Virus of rabies
Neisseria gonorrhoeae	Virus of poliomyelitis
Neisseria intracellularis (some strains)	Virus of influenza
Pasturella multocida	
Hemophilus pertussis	
Hemophilus influenzae (some strains)	
Treponema (some strains)	
Clostridia	
Brucella suis	
Brucella melitensis	

employed at the time of its introduction. For example, 25 strains of *M. pyogenes* that were resistant to penicillin, oxytetracycline, chlortetracycline, chloramphenicol, or streptomycin were as sensitive to erythromycin as were nonresistant strains. When strains of this organism were made resistant to erythromycin, they did not develop increased resistance to other antibiotics. In fact, on becoming resistant to erythromycin some strains became more sensitive to one or more of the other antibiotics. Later, however, definite evidence of cross resistance between erythromycin and carbomycin was reported by Fusillo and his associates.[13] It is interesting that Haight and Finland [14] found that erythromycin exerted its effect primarily on multiplying bacteria, as does penicillin. That the effect of erythromycin on the growth rate of *M. pyogenes* varied according to the concentration of the antibiotic was evident from the report of Hobson.[15] It was further evident from his report that the development of resistance to erythromycin by staphylococci appears to follow a stepwise pattern resembling that seen with penicillin.

In a study on antibiotic synergism Rantz and Randall[16] reported that there did not appear to be any synergism between erythromycin and penicillin or oxytetracycline or chloramphenicol. The fact that no synergism was demonstrated, however, does not infer incompatibility. It was evident, for example, from the report of Powell and his associates[17] that erythromycin appeared to be compatible with a number of antibiotics. They further showed that there was definite synergism between erythromycin and sulfadiazine. These same investigators also carried out studies in which an erythromycin-penicillin compound was used in the treatment of hemolytic streptococcal infections in mice. The results were compared with those in a similar experiment in which erythromycin and penicillin were used as combined (two-drug) therapy. The results in the two experiments were essentially the same. In other words, there appeared to be no advantage in using a chemical combination of penicillin and erythromycin over using the two antibiotics, as is commonly done, in combined therapy.

Chapter III

Absorption, Diffusion, and Excretion of Erythromycin

It was evident from the very early studies that erythromycin is readily absorbed and diffuses fairly well throughout most body tissues. It appears to be excreted fairly readily in the bile, urine, and feces. There are four methods of bioassay that have been employed most commonly by investigators of this general subject.

Methods of Assay

These methods of assay include the cup-plate agar diffusion method, various modifications of the serial dilution technique, the turbidimetric method, and the so-called slide-cell method. Best results will be obtained by whichever method is found most suitable by the individual investigator.

Cup-Plate Method. This method was described by Kirshbaum and his colleagues[18] and in their opinion yielded results that were within the inherent error of the biologic method. The procedure is as follows:

The cylinders (cups) used in the assay have an outside diameter of 8 ± 0.1 mm., an inside diameter of 6 ± 0.1 mm., and a 10 ± 0.1 mm. length.

Nutrient agar for both base and seed layers has the following composition:

Peptone (B-B-L Selysate) (Bacto)	6.0 Gm.
Pancreatic digest of casein (B-B-L Trypticase) (Bacto-Casitone)	4.0 Gm.
Yeast extract	3.0 Gm.
Beef extract	1.5 Gm.
Glucose	1.0 Gm.
Agar	15.0 Gm.
Distilled water to make up to	1000.0 ml.
Adjust so that the *p*H is 8.0 after sterilization	

The erythromycin working standard is kept at room temperature under

desiccation. A stock solution containing 1000 μg./ml. in 0.1 *M* phosphate buffer *p*H 7.0 is prepared, which may be kept for one week under refrigeration (about 10 C.) and for a much longer period at —20 C. (Erythromycin goes into solution more easily if it is first dissolved in a small quantity of ethyl alcohol.) In the test this stock solution is further diluted to 2.0 μg./ml. in 0.1 *M* phosphate buffer *p*H 7.0 and then to 0.2 μg./ml. with sterile 7.0 per cent bovine albumin (fraction V) in 0.1 *M* potassium phosphate buffer. The *p*H of the bovine albumin solution is adjusted to 7.4 prior to use in the dilution.

In preparing the plates 10 ml. of agar is added to 20×100 mm. Petri dishes and allowed to harden. This constitutes the "base layer." The organism suspension is made as follows: Transfer *Sarcina lutea* (P. C. I. 1001, American Type Culture Collection), the test organism, to an agar slant and incubate 24 hours at 26 C. Wash the growth off with about 3.0 ml. of nutrient broth and spread over the surface of a Roux bottle containing 300 ml. of agar. Incubate for 24 hours at 26 C. and wash the growth from the Roux bottle with 15 ml. of nutrient broth. This bulk suspension, when diluted 1:10 should have a light transmission of 10 per cent when tested in a photoelectric colorimeter with a 6500 Å. filter. Add 0.2 ml. of this suspension for each 100 ml. of agar, which has been melted and cooled to 48 C. Add 4.0 ml. to each plate containing 10 ml. of the uninoculated agar (base layer) and spread evenly over the surface. The Petri dishes are covered with porcelain lids glazed on the outside. Place six cylinders on the inoculated agar surface, when hardened, so that they are at approximately 60 degree intervals on a 2.8 cm. radius.

In preparing the standard curve, dilute the 0.2 μg./ml. solution in bovine albumin to 0.02, 0.04, 0.06, and 0.1 μg./ml. in the same diluent. From the 2.0 μg./ml. concentration in phosphate buffer make 0.4 and 0.8 μg./ml. in bovine albumin.

Use three plates for the determination of each point on the curve, a total of 18 plates. On each of three plates fill three cylinders with the 0.2 μg./ml. standard and the other three cylinders with the concentration under test. Thus there will be 54 of the 0.2 μg./ml. determinations and nine determinations for each of the other points on the curve. After the plates have been incubated, read the diameters of the circles of inhibition. Average the 54 readings of 0.2 μg./ml. concentration and the readings of the point tested for each set of three plates. The average of the 54 readings of the 0.2 μg./ml. concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 0.2 μg./ml. reading for that set of three plates were the same as

the correction point. Thus, if in correcting the 0.06 unit concentration, the average of the 54 readings of the 0.2 μg./ml. concentration is 18.3 mm., and the average of the 0.2 μg./ml. unit concentration of this set of three plates is 18.9 mm., the correction is 0.6 mm. If the average reading of the 0.06 μg./ml. concentration of these same three plates is 15.4 mm., the corrected value is then 14.8 mm. Plot these corrected values, including the average of the 0.2 μg./ml. concentration, on two-cycle semilogarithmic paper, using the concentration in μg./ml. as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points.

Use one plate for each unknown sample. Fill three cylinders on each plate with the standard 0.2 μg./ml. solution and three cylinders with the undiluted serum sample, alternating standard and sample. Incubate the plates for 16 to 18 hours at 26 C. and measure the diameter of each circle of inhibition. To estimate the concentration of the sample, average the zone readings of the standard and the zone readings of the sample on the one plate used. If the sample gives a larger average zone size than the average of the standard, add the difference betwen them to the 0.2 μg./ml. zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 0.2 μg./ml. value on the curve. From the curve read the concentrations corresponding to these corrected values of zone sizes.

Serial Dilution Technique. This method for the assay of erythromycin in body fluids was described by Ziegler and McGuire.[19] According to them, the reproducibility of the method is quite satisfactory. It is a modification of the Rammelkamp method[20] for determining the concentration of penicillin in body fluids.

The assay medium is composed of a beef infusion broth to which is added 5.0 per cent defibrinated blood as a source of red blood cells. In their laboratory defibrinated sheep blood is employed routinely, but defibrinated blood from certain other animals may be substituted. Human blood (type O) has been employed successfully in media for the assay of antibiotic concentrations in human sera. Rabbit blood has proved to be satisfactory for the antibiotic assay of sera of various animals, but the destruction of complement in the sample may be required. Dog blood is an unsatisfactory source of red cells for this assay procedure because of the fragility of the red cells.

The test organism is a beta hemolytic streptococcus, strain C203 (ATCC 8668), which is maintained in a virulent state by mouse passage. Frequent mouse passage is not essential, for the test organism has retained its hemo-

lytic activity and sensitivity to erythromycin for a period of two months without animal passage.

A primary culture of the test organism is prepared by the inoculation of 10 ml. of blood-infusion broth, with blood from the heart of an infected mouse. All cultures of the test organism are carried in beef-infusion broth (9 ml. per tube) supplemented with defibrinated sheep blood (1 ml. per tube), and incubation is carried out at 17 C. for six to eight hours. Inoculation of a secondary or working stock culture is made with 0.5 ml. of the primary culture. Following incubation at 37 C. for six to eight hours, the culture may be held in a refrigerator for two weeks.

A daily inoculum culture is prepared by the inoculation of 9 ml. of 10 per cent blood-infusion broth, with 1 ml. of the working stock culture, followed by incubation at 37 C. for six hours. Growth of the culture is indicated by hemolysis of the red blood cells. The culture prepared in this manner is diluted 1:100 with broth. An inoculum of 0.04 ml. of this diluted culture is added to each assay tube.

A standard stock erythromycin solution containing 1000 μg./ml. is prepared by dissolving the antibiotic material in 70 per cent ethanol. The reference standard thus prepared may be stored for one week at 5 C. A working standard containing 5.0 μg./ml. is made up daily by diluting the stock solution with sterile 0.85 per cent saline solution.

Blood samples submitted for assay are stored at 5 C. until the normal clot retracts. With aseptic procedures the clot is broken, the samples are centrifuged, and the supernatant serum is pipetted into the assay tubes. Slight discoloration of serum due to hemolysis does not interfere with the assay.

Normal serum often brings about spontaneous hemolysis of red cells of the test medium in uninoculated control tubes. This effect may be eliminated by the destruction of complement by heating the serum at 62 C. for three minutes. This treatment does not result in detectable destruction of antibiotic activity. Likewise the antibiotic activity of erythromycin is not diminished by the effects of serum constituents. When solutions of known concentrations of erythromycin are prepared in whole serum, the antibiotic activity is neither destroyed nor inactivated by adsorption on the serum constituents.

Estimation of erythromycin concentrations in tissues of experimental animals also has been carried out successfully by this assay method. The tissues are excised, weighed, and homogenized under aseptic conditions. The resulting homogenates may be centrifuged and assayed in the same manner as serum samples.