THE DOSAGE SCHEDULE OF CHEMOTHERAPEUTIC AGENTS

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We shall define a chemotherapeutic agent as a drug used in treatment of a systemic infection. Although Ehrlich, in addition, limited the term to drugs of known chemical constitution in order to exclude antitoxins, serums and vaccines, we shall not adopt this restriction since antiparasitic agents of microbial origin which may be of unknown constitution at present play an important role in therapy.

In the investigation and use of any chemotherapeutic agent, there is a triad of three factors which must be taken into account. These are the parasite, the host, and the drug. It is the interplay of these three factors which accounts for the cure of the infection. An understanding of these factors demands not only a study of the effect of the drug on the parasite *in vitro* and *in vivo*, but also an investigation of the absorption, excretion, degradation and distribution of the drug in the host organism.

In designing the most satisfactory dosage schedule for any chemotherapeutic agent at least three factors must be considered in connection with the effect of the drug on the parasite *in vivo*. These are the total dosage, the duration of treatment, and the interval between administration of individual doses. In any type of treatment an unlimited number of variations with respect to total dosage, duration of treatment, and interval between doses are possible. Unless experiments are designed properly, one cannot be certain whether total dosage, duration of treatment, or spacing of the individual doses is responsible for the better therapeutic effect.

The interval between doses should be determined by knowledge of whether or not constant contact between chemotherapeutic agent and parasite is necessary for most effective therapy and by a knowledge of the absorption, distribution, excretion and degradation in the host of the particular drug in question. It would appear that each individual drug, and possibly each infection for which it is used, must be studied in order to determine whether or not a dosage schedule which attempts to maintain a constant concentration of drug in contact with the parasite is necessary for the most effective therapy. Spacing the total dosage may involve a large initial loading dose followed by smaller maintenance doses. This may be necessary for one of two different reasons; namely, to establish rapidly equilibrium in the body with the drug or to protect from the infection until subsequent doses can be given. The large initial doses recommended with the sulfonamides and quinacrine are examples of the former, while a large initial dose of penicillin in a mouse infection (109) serves as an example of the latter.

The evidence appears very strong that in certain instances most effective therapy is obtained by rapidly attaining and then maintaining an effective concentration of drug in blood for a certain time, while with other drugs just as efficient or even more efficient therapy may be obtained when the concentration of drug

in the blood is not maintained constant but is allowed to rise to a peak and then fall to a negligible level before the next dose of drug is given. It will be shown that sulfonamides are most effective when given by continuous dosage while penicillin is just as effective in the same infections when given intermittently. One might explain this difference by assuming the sulfonamides to be more loosely bound to receptors in bacteria than penicillin (for discussion, see Marshall, 57). It should be pointed out that *in vitro* studies alone can never serve as a basis for devising the most efficient dosage schedule, but in addition it is necessary to have knowledge of the behavior of the drug in the host, and direct studies of dosage schedules in infected animals. Then, trial in diseased human beings can be undertaken to see if a dosage schedule, based on the knowledge obtained, is satisfactory. In patients, investigation of dosage schedules is much more difficult and less accurate than in rigidly controlled experiments on infected animals. There are added difficulties in patients with a disease having a high mortality. If some dosage schedule has been tried on patients and found more or less satisfactory, it is very difficult to attempt to modify it.

In 1917, Moore and Chesney (69) in a study of the value of ethylhydrocuprein in pneumonia in man utilized the acquired bactericidal power of serum to determine the best dosage schedule. Two quotations will be given from their paper as their dosage schedule of this drug resembles very closely the schedule adopted for the sulfonamides but antedates that schedule by more than two decades. They state: ". . . what is the optimum method of administration of the drug, both as regards the route, and the regulation of the size and spacing of the individual doses, as determined by a study of this acquired bactericidal property of the serum?" ". . . in order to destroy pneumococci within the living animal, the drug should be repeatedly administered in suitable doses, at comparatively short intervals of time, in order that the pneumococcidal action in the circulating fluids may be continuously preserved for the necessary length of time." It may be noted that in the dosage schedule used by Moore and Chesney a large dose was first given to attain quickly a bactericidal action of serum and then smaller doses were given at frequent intervals in order to maintain this bactericidal action. The use of both priming and maintenance doses has been later found necessary for many chemotherapeutic agents. It is interesting that these authors did not write of the ethylhydrocuprein concentration in blood but used the term "acquired bactericidal property," which described exactly what they were measuring. This was in contrast to what happened with penicillin, where results of drug concentration in blood obtained by a non-specific, easily influenced biological method were used as if they were obtained by a highly specific and accurate chemical method. In this study of ethylhydrocuprein, no evidence was available that the maintenance of a bactericidal property of serum was essential for the most effective method for use of the drug. However, even today, unless evidence were available to the contrary, the dosage schedule used by Moore and Chesney would appear to be the one of choice as a first attempt to discover the value of a chemotherapeutic drug. Subsequent investigation would then be made to determine the optimal dosage schedule.

We can now consider the dosage schedule of arsenic and antimony compounds. Ehrlich attempted a *therapia sterilisans magna* where one dose of drug would completely eradicate the infection. When this failed with arsphenamine in human syphilis, the dosage of this arsenic compound was given in a more or less empirical manner and not based on any carefully controlled experimental study. Only twenty to thirty years after their introduction, do we find a change in dosage schedule of the arsenicals. Hyman *et al.* (46) introduced the so-called massive dose chemotherapy for syphilis by the intravenous drip method, where over only a five day period a large dose of neoarsphenamine (and later oxophenarsine) was given by slow intravenous drip. This 5-day treatment of early syphilis was apparently quite effective and nullified the syphilographer's idea of the necessity for treatment for more than a year. However, the short intensive method proved to be disadvantageous from the standpoint of toxicity. The published results did stimulate Eagle and Hogan to undertake an experimental study in syphilitic rabbits of the dosage schedule of oxophenarsine (30). From their studies, it appears that the curative total dose of oxophenarsine in rabbit syphilis does not vary greatly despite wide variations in the duration of treatment and frequency of injections. The data presented in support of this conclusion are not too impressive and one cannot accept the author's statement that the data prove that a high concentration of drug in body fluids for a short period of time was more effective therapeutically than a low concentration maintained for a relatively long period. This work of Eagle and Hogan does not contain a single experiment in which the concentration of drug in blood was maintained for more than a fraction of the twenty-four hours. The final evaluation of "cure" at six months was only made on a proportion of the animals used. The correction used on animals followed for only six weeks is subject to a large error. In addition, there are very large probable errors in data of this kind with the small number of animals used. The authors did not seem to appreciate this and made no attempt to estimate these errors.

It has also been found that the curative dose of reduced tryparsamide for a trypanosome infection in mice is the same for intravenous, subcutaneous, and intraperitoneal injection. From these data, it is concluded that a relatively high concentration of drug for a short period is equally as effective as a relatively low concentration for a longer period (42). It is probable that with the organic arsenicals in the therapy of syphilis and of trypanosomiasis, the total dosage is more important than the manner in which the dosage is divided. This means that a constantly maintained concentration of drug in the blood is no more effective than allowing intervals during treatment where the drug has disappeared from the blood.

Vianna (103) in 1912 discovered the curative effect of antimony in mucocutaneous leishmaniasis, and Di Cristina and Caronia (24) three years later found a marked reduction of mortality in kala-azar by administering tartar emetic by intravenous injection on alternate days. The practical consequences of this discovery on a disease which hitherto had a mortality of 95 per cent were extraordinary. Although a cure rate of over 80 per cent was attained, the imperfections of

the lengthy tartar emetic treatment led to the search for a more intensive and less unpleasant form of treatment. The pentavalent antimonials made it possible to reduce the number of injections from 30 to 10, but treatment was still given on alternate days. The toxicity of the preparations used apparently conditioned the dosage schedule, as daily injections were tried when less toxic preparations became available (37). In the experimental disease in the hamster, it has been found that injections of pentavalent antimonials given daily were less effective than an equal number given bi-weekly, the total dosage remaining constant (104). However, in both the human and the experimental disease, variation in the number of injections has been accompanied by variation in the duration of treatment. Stilbamidine, which has proved effective in the case of antimony-resistant kala-azar, has been given intravenously daily or even at less frequent intervals. However, the total dosage, the number of injections and the duration of treatment have all been varied (86).

The non-metallic and extremely interesting compound suramin, which has been shown to be highly effective in the early stages of trypanosomiasis is retained in the body for such a long time that dosage can be given at very long intervals. In fact, a single intravenous dose is claimed to protect for several months. There is no mystery about this prolonged action, as the drug remains in the blood for many months (38).

There are three features in connection with the sulfonamides which were responsible for the development of rapid, accurate, and fairly specific methods for their chemical determination in body fluids and tissues (60, 41, 55, 9, 106). These are the use of relatively large dosage, the nature of the distribution of these drugs in the organism, and the fact that all active drugs of this group possess an aryl-amino-group or are converted in the body to a compound containing this group. Such compounds containing an aryl-amino-group can easily be diazotized and coupled to form a highly colored azo-dye. The use of these simple methods for the determination of sulfonamides led to the development of the dosage schedule necessary to obtain the optimal therapeutic response. The concepts which have guided therapy with this class of drugs are a) the use of the concentration of drug in blood or plasma rather than dosage by mouth to control therapeutic response, and b) the demonstration that the best therapeutic effect is obtained by raising the drug concentration in blood to the desired level rapidly by giving a large loading dose and then giving the drug at frequent intervals, day and night, so as to maintain this concentration in the blood for several days. Let us now inquire into the evidence for the validity of these concepts.

In regard to the first, it may be said that the sulfonamides which have had clinical use have an apparent distribution of from 45 to 80 per cent of the body weight. This means that within a factor of two, at most, the drug concentration in plasma is proportional to the total amount of drug in the body with the various sulfonamides. Since some of these drugs are incompletely and erratically absorbed from the gastrointestinal tract and different ones are excreted at different rates by the kidney, the amount of drug in the body is represented better by its concentration in plasma or blood than by dosage by mouth. Granting that therapeu-

tic activity is related to the amount of drug in the body (exclusive of that in the gastrointestinal tract), it follows that for these drugs the concentration in blood should correlate with chemotherapeutic activity. In fact, soon after their introduction, sulfonamides were used in therapy on the basis of concentration in blood rather than on dosage administered.

In regard to the second concept of the necessity for maintaining a more or less constant concentration of drug in the blood in order to obtain the optimal therapeutic response, it must be admitted that published data are rather scanty in support of this thesis. However, there is little doubt that it is, on the whole, correct. In pneumococcal infections of mice, sulfanilamide was stated by the earlier workers (12, 50, 34) to have no beneficial effect. However, in all the above experiments doses of 5 to 25 mgm. per mouse were given once a day. Schmidt (81, 82) found that if 5 or 10 mgm. of sulfanilamide were given every 4 to 6 hours, a marked curative effect was observed on many types of pneumococcal infections of mice. Litchfield, White and Marshall (48) also found sulfanilamide very effective in a pneumococcal infection of mice when the drug was administered by the drug-diet method. Also it was found that 4,4′-diaminodiphenylsulfone was twenty to one hundred times as active as sulfanilamide in a streptococcal infection of mice when the drugs were given once a day, but it was only about three times as effective when the drugs were administered by the drug-diet method. The difference appears to be due to the slower absorption and excretion of the sulfone as compared to sulfanilamide (61). It has also been found that in an infection of mice with *E. coli*, sulfathiazole administered in 5 doses (at 2-hour intervals) was more effective than a single dose, or, in other words, the long duration of a low concentration of drug in blood is more effective than a short duration of a high concentration (108). In addition, in lophurae malaria in the duck, the sulfonamides are much more effective therapeutically when the concentration in blood is maintained more or less constant than when intermittent doses are given.

Thus, after the sulfonamides had been established as effective agents in bacterial infections, a number of clinical and experimental studies were undertaken to test their efficacy in malaria. These were contradictory partly on account of a species difference in the susceptibility of parasites and partly due to the different dosage schedules which were used. It was found that the maintenance of a constant concentration of a sulfonamide in the blood for a sufficient length of time is just as important for effective therapy of lophurae malaria in the duck as it is in bacterial chemotherapy. When these drugs were administered to ducks with lophurae malaria by the drug-diet method so as to maintain a more or less constant concentration of drug in the blood, it was found in every case that more drug was required to produce the same therapeutic response when given as a single daily dose than when administered in the diet. Thus 2.6 times as much sulfanilamide, 10.5 times as much sulfaguanidine, and more than 40 times as much sulfathiazole were required with single doses per day as were needed with continuous administration (62).

For a long time the only effective chemotherapeutic agent for use in malaria was quinine. Usually, it was given at first 1 or 3 times a day, but occasionally was

administered so as to try and maintain the amount of alkaloid in the body as constant as possible. Thus, MacGilchrist (51) in comparing the relative therapeutic value of several cinchona alkaloids in malaria gave a dose of medicine every eight hours throughout the twenty-four hours of the day so as to keep the amount of alkaloid in the body as nearly constant as possible. Others (26) have given doses of quinine every three hours from 6:00 A.M. to 9:00 P.M. with apparently the same object in view. The reason for attempting to maintain a more or less constant concentration of drug in the blood in treating malaria may have been the desire to have an adequate concentration present at sporulation if this was irregular as a result of several superimposed infections. The belief that quinine is effective only during sporulation was widely held by many, but others held the dogma that quinine is only effective on young schizonts. There was little or no scientific evidence available on the question.

Experimental malarial infections with avian parasites were used for testing the efficacy of new drugs in 1926. The dosage schedule in common usage for many years was that of Roehl (80), *i.e.*, the administration *per os* of one dose of drug per day for 6 days. There was no information as to whether this was the optimal method of therapy. It is of interest to recall that in 1914 Marks (53) had found that methylene blue was therapeutically effective when fed in the diet but ineffective when given in single daily dosage in an avian malarial infection. Presumably, the diet method prolonged the persistence of the drug in the blood.

Quinine is more effective when given in a single daily dose than in divided doses or by continuous intravenous injection in lophurae malaria of the duck (58, 22, 8). However, the reverse is true in cynomolgi malaria in the monkey where it has been shown that 4 doses per day for 7 days are three times as effective as a daily dose for the same time (13). It should be noted that in lophurae malaria the effectiveness was based on reduction of parasitemia, while in cynomolgi malaria cure of the disease was the criterion used. This shows clearly that not only the particular drug but the species of parasite and probably the host must be taken into consideration before generalizations as to dosage schedules can be made. Obviously, a more or less constant concentration of drug in the blood is not always the most effective method of therapy in all malarias and with all drugs.

In the elaborate search for new antimalarial drugs carried out in this country during World War II (93), it was assumed for human malarias from analogy to the sulfonamides that 1) the antimalarial action of a drug is related to its concentration in the plasma, and 2) that a dosage schedule giving a more or less constant concentration of drug in plasma furnishes the most effective therapy (89, 2). However, there is no unequivocal evidence that either of these assumptions is true for human malaria for all drugs. Let us consider dosage schedules of the two drugs which have been used and studied most in human malarias, namely, quinine and quinacrine. These two drugs differ markedly in their physiological disposition. Quinine is accumulated in the tissues to a very limited extent and is rapidly excreted and degraded. On the other hand, quinacrine is highly localized in the tissues, many tissues containing several thousand times the concentration present in plasma, and persists in the organism for a long time. It appears that

in the case of quinine, the concentration of drug in the plasma represents fairly accurately the amount of drug present in the organism, while in the case of quinacrine the concentration of drug in plasma can vary widely with a constant total amount of drug in the body. In fact, the total amount of quinacrine present in plasma is extremely minute compared to the total amount in the body.

In the duck, it has been found that with a constant dosage, the concentration of quinacrine in the plasma is highly variable in different animals or in the same animal at different times. These variations in concentrations of drug in plasma are caused mainly by variations in the distribution ratio of quinacrine between plasma and tissues and not by variations in the total amount of drug in the organisms, as is the case with the sulfonamides. Moreover, it has been shown that variations of concentration of drug in plasma are not correlated with the concentration of drug in erythrocytes or in the malarial parasites. In the duck antimalarial activity is well correlated with the dosage of quinacrine and with the total amount of drug in the host, but very poorly or not at all correlated with the drug concentration in plasma (59).

In man there are no data on variations in the distribution ratio of quinacrine between plasma and tissues; it has been assumed in man that the concentration of quinacrine in the plasma is correlated with the concentration of quinacrine in tissues and with therapeutic activity (90, 25, 33, 87). However, in the case of the rabbit and of the dog it has been shown that variations in concentrations of quinacrine in plasma produced by the same dosage are not correlated with concentrations of drug in the tissues; here, as in the duck, the distribution ratio between plasma and tissues is highly variable (23). In addition, when quinacrine is used to treat blood-induced infections of cynomolgi malaria in the monkey, it has been found that the percentage of cures is directly proportional to the dosage given but is not at all correlated with variations in the concentrations of drug in plasma produced by the same dose (83).

In the case of quinine, therapeutic activity in vivax malaria appears to be correlated with variations in concentrations of drug in plasma produced by the same dosage (88). Quinine, although apparently concentrated to some extent in tissues, resembles the sulfonamides more than it does quinacrine in its distribution pattern.

Quinine was usually given before World War II on a dosage schedule of three times a day for therapeutic effect and once a day for suppressive action. The therapeutic dose may have been split in order to avoid toxicity from a large single dose. In studies during the War, it was given so as to achieve a fairly stable concentration in the plasma (94). There is however, no evidence that I know of, to show that in human malarias a fairly constant concentration of quinine in plasma is more or less effective than concentrations which fall below the effective level for a considerable period of time. The dosage schedule recommended today is the administration of a dose three times daily (18).

The dosage schedule proposed and used for quinacrine until World War II was administration of 0.1 gram three times a day. Its action in controlling an attack of malaria was found to be slower than that of quinine. Bryant (10) in 1942

published observations which he had made in East Africa for several years before the War. Noting that the fever decreased rapidly when the urine became bright yellow (third or fourth day of quinacrine administration) and believing this to be due to the exceeding of a renal threshold of the drug in plasma, he administered large doses of quinacrine on the first and second days of treatment. He found that the urine became yellow and the fever receded on the first or second day. Findlay *et al.* (39) also used loading doses of quinacrine in treating malaria in West Africa partly on an empirical basis and partly as a result of Bryant's work. Shannon *et al.* (90) before the observations of Bryant were known made a careful study of the pharmacology of quinacrine. As a result of this study, they proposed an entire revision of the dosage schedule of quinacrine for most efficient therapeutic and suppressive therapy. This is the dosage schedule recommended for quinacrine today, a large loading dose and a maintenance dose thereafter (18).

When penicillin[1] was introduced for the treatment of bacterial infections, it was assumed that most effective therapy required the maintenance of a constant concentration of drug in plasma, day and night, for a sufficient length of time. There was no evidence at all that this idea was correct; it was, indeed, a transference to penicillin of a concept which had been established with the sulfonamides. Due to the extremely rapid excretion of penicillin by the kidneys, it was at first thought necessary to give a continuous intravenous injection of the drug to attain effective therapy. However, soon after its introduction, the continuous intravenous drip was abandoned in favor of intramuscular injections at 2- to 4-hour intervals. Due to the inconvenience of this dosage schedule to both patient and attendants, various methods have been used to prolong the effective concentration in the plasma that results from a single injection of penicillin. These methods decrease the rate either of its absorption from muscle or of its excretion by the kidneys. For decreasing absorption various preparations have been used, for example, a suspension of penicillin in peanut oil and beeswax or a suspension of the procaine salt of penicillin in either oil or water (depositary preparations). To decrease excretion by the kidneys, two drugs have been used, carinamide (4) and Benemid (7).

Despite this fact that most dosage schedules of penicillin seem to have been designed with the objective of maintaining the plasma concentrations of this antibiotic at or above levels which are known *in vitro* to inhibit the growth of the invading microorganisms, an examination of the background of this concept shows that it is not supported by established fact (56). In 1944, Fleming and coworkers (40) wrote: "We do not yet know whether it is better to maintain a constant low level of penicillin in the blood, or to have a very high level for a short time after an injection, followed by a period of very low level before the next injection. Clinically both systems have worked excellently. It will take a long series of observations to decide which is the better." In the same year, Tillett and coworkers (95, 96) working with rather crude preparations of penicillin showed that pneumococcal lobar pneumonia could be treated satisfactorily when

[1] Where penicillin is used, it refers either to the original crude preparation or to benzylpenicillin (penicillin G). Other species are appropriately designated.

an interval of 12–16 hours was allowed to elapse between courses of injections of penicillin. At about this time others (21, 64) questioned the necessity of maintaining an effective concentration of penicillin in the blood as is the custom with sulfonamide therapy. In spite of lack of evidence for the concept of a maintained concentration of drug in blood, almost all penicillin therapy is still today based on preparations and dosage schedules which attempt to maintain an effective concentration of drug in plasma. Acceptance of this concept has been so complete that at present the administration of penicillin is directed toward the continued maintenance of a certain concentration of this drug in plasma rather than towards the most efficient therapeutic regimen.

The whole question of the most efficient dosage schedule for penicillin is complicated by the fact that no chemical method has been devised for determining the concentrations of penicillin in plasma and body fluids. Hundreds of reports giving the concentrations of penicillin in plasma, urine, body fluids, and even in tissues have appeared, but what is really being determined is the bacteriostatic power of body fluids and tissues after the administration of penicillin. This is exactly what Moore and Chesney (69) determined after administration of ethylhydrocuprein more than a third of a century ago. However, these authors did not write of the ethylhydrocuprein concentration in serum but used the term "acquired bactericidal property" which described exactly what they were measuring. It is gratifying to find at least one author who writes of the "antibacterial activity of serum" instead of concentration of penicillin (40), and another who states: "No attempt has been made to express the penicillin content of the specimens examined as units of penicillin per ml. of fluid, since at the present time this is not possible without certain assumptions." (63). This latter worker also tested the antibacterial activity of the serum or body fluid before the administration of penicillin and frequently found some activity. This control has been neglected by practically all other workers.

Probably many factors influence the bacteriostatic action of penicillin *in vitro*. One at least has been fairly carefully studied. This is the effect of serum or albumin on the activity of penicillin. It has been found (15, 78, 97) that penicillins G, X and dihydro-F are bound to the extent of fifty to sixty per cent in normal plasma, while penicillin K is at least ninety per cent bound. In using the microbiological method for the assay of penicillin in serum by an ordinary serial dilution of serum, bacteriostasis will occur in the tube where the unbound concentration of drug, not the total concentration, is at a level which just prevents growth. This introduces a very serious error in assays, particularly at low concentrations of drug; and, in fact with low concentrations of penicillin K in serum, there is a correction of more than tenfold (27). Various devices have been used in an attempt to avoid this protein error, but as Davis (20) pointed out, "Even with the best possible technic, the determination in serum of low concentrations of penicillin, especially K, involves considerable error. All of the earlier assays for penicillin, including Eagle's extensive studies on the rate of destruction of the various penicillins in the body, must be reevaluated in the light of the knowledge that an uncorrected assay gives erroneously low values; and since this error is greater at

lower concentrations of drug, the reported rates of disappearance have been greater than the true rates, especially with penicillin K." Tucker (99) has reported that when penicillin is added to spinal fluid (containing only a trace of protein) it cannot be completely recovered by the serial dilution method of assay.

In spite of the fact that determinations of the concentration of penicillin in serum by the microbiological method are questionable, one can from carefully controlled experiments in infected animals arrive at some decision as to the most effective dosage schedule of penicillin. In addition, one can use the unsatisfactory microbiological method to decide if it is necessary to have an effective concentration of penicillin continuously in contact with the microorganism for most effective therapy. A number of such experiments have been reported. In 1946, Jawetz (47) showed that, where measurable blood concentrations persisted for only one hour after injection of penicillin, the depression of bacterial population in a streptococcal infection of mice persisted for at least eight hours. A number of investigators have studied different dosage schedules of penicillin in experimental streptococcal and pneumococcal infections. In some of these studies, the interval between injections was varied, with or without varying the number of injections, but without keeping the total time of treatment constant. The total time of treatment may be more important than the number of injections. Zubrod (109) studied various schedules of penicillin dosage against a hemolytic streptococcal infection of mice and found that cure of the infection could be obtained when penicillin was given at widely spaced intervals. Eight doses at 3-hour intervals were no more and probably were less effective than three doses at 8-hour intervals. This investigator emphasized the importance of a large initial dose regardless of what schedule is adopted thereafter. The advantage consists in the fact that a large initial dose protects mice for the next twenty-four hours, whereas with multiple dose schedules, many mice die before they receive enough drug to bring the infection under control. In this same streptococcal infection White, Baker and Jackson (107) treated mice immediately after infection for a period of twenty-four hours with different dosage schedules of penicillin X. They showed that under these conditions, 1, 2, 4 and 8 doses were equally effective in terms of the total amount of penicillin necessary for the median survival dose for each schedule. Gibson (43) states that the total curative dose of penicillin administered for four days to mice infected with pneumococci was the same whether injections were made at 12- or at 3-hour intervals. However, the number of animals used was small. On the other hand, Eagle, Fleischman and Musselman (29) studied in a pneumococcal infection of mice and in a streptococcal infection of rabbits the effect of varying the intervals between a fixed total number of injections of penicillin. As the interval between the injections was progressively increased, the median curative dose at first decreased to reach a minimum and then increased. Since here the duration of treatment as well as the spacing of injections was varied, the results do not help to clarify the problem of continuous versus intermittent therapy.

Miller *et al.* (68) have studied the dosage schedule of penicillin in an experimental infection in mice with an organism of high penicillin resistance, *Salmonella*

typhosa. They found that a smaller total amount of penicillin was required for protection when treatment was given as a single injection than when 2, 3, 6 or 12 doses were given in a twenty-four hour period. When the time interval between two doses was varied from 2 to 36 hours, it was found that the two dose 2-hour schedule required even a smaller total amount of penicillin than the single dose.

In addition to these studies on mice, we have two extensive and carefully controlled studies on pneumococcal infections of rats. In the first study, Schmidt, Walley and Larson (85) infected rats intraperitoneally with pneumococci and initiated therapy with crystalline penicillin four hours later, at a time when bacteremia ranged from 2000 to 80,000 organisms per cc. The data showed that cure of the infection can be accomplished by the administration of penicillin at either frequently spaced or widely spaced intervals. With treatment of brief duration, two doses of penicillin administered 2 or 4 hours apart were more effective than a single dose or two doses separated by an 8-hour interval, the two doses at a 4-hour interval being the most effective. When a constant period of treatment (96 hours) was used, doses administered at 2-, 4- and 8-hour intervals were equally effective and were more effective than doses at twelve or twenty-four hour intervals. It was also found that procaine penicillin administered at 24-hour intervals was more effective than aqueous penicillin given at 12- or 24-hour intervals but less effective than the aqueous penicillin given on a 2-, 4-, or 8-hour schedule. Measurements were made of the concentration of "penicillin" (bacteriostatic substances) present in plasma after equally effective doses of penicillin on the various dosage schedules. These measurements showed that these equally effective doses maintained inhibitory concentrations of "penicillin" in plasma for only a small fraction of the dosage interval. In the second study, Schmidt and Walley (84) used the same pneumococcus to produce a lobar pneumonia in rats. Here, the animal infection resembles human infections more closely than the fulminating peritonitis usually used. The duration of treatment and dosage schedules were the same as in the first study. This study is of importance in showing that intermittent administration of penicillin, where long periods elapse without a bacteriostatic concentration in plasma, is more effective or takes less total penicillin than in schedules with more frequent dosage. As, in the opinion of the reviewer, this is by far the best and most important study on the dosage schedule of penicillin, the conclusions of the authors are given. "The results of the studies outlined above have shown (1) that the effectiveness of sodium penicillin G in the treatment of established lobar pneumonia is determined by the frequency with which the drug is administered; (2) that by all criteria, sodium penicillin is considerably more effective when administered at 8-hour intervals than when given in either more frequent or more widely spaced doses; (3) that with this most effective dosage schedule, bacteriostatic concentrations of 'penicillin' are maintained in plasma for only a small fraction of the treatment interval; (4) that with respect to either individual or total treatment periods, there appears to be no direct relation between the effectiveness of a regimen and the length of time during which the plasma contains bacteriostatic concentrations of 'penicillin'; (5) that the effectiveness of a dosage schedule appears to be a product of both the height

of the concentration of 'penicillin' in plasma and the duration of detectable concentrations; and (6) that the most effective schedule for administering sodium penicillin G is in every respect superior to the 24-hour administration of the allegedly long acting procaine penicillin."

Although the above findings show that to some extent the effectiveness of penicillin therapy is related to the frequency of treatment, they offer no support for the general assumption that the effectiveness of therapy is dependent upon the continued maintenance of inhibitory concentrations of drug in plasma. Rather, the experimental results indicate that optimal therapeutic effects may be achieved by administration of aqueous penicillin at fairly widely spaced intervals and in fact there is a definite suggestion that intermittent therapy with aqueous penicillin may be more effective than frequent dosage with aqueous penicillin or the use of depositary preparations.

Reports dealing with human infections have proved the efficacy of penicillin therapy which does not maintain an effective concentration in plasma. In pneumococcal pneumonia administration of aqueous penicillin once or twice a day is apparently just as effective as administration at frequent intervals (101, 74, 45, 105). In surgical infections, intermittent administration appears to be satisfactory (1). Streptococcal sore throat has been treated successfully with an intermittent dosage schedule. There were much fewer patients with significant antistreptolysin response in a group on a three hour than in one on a twenty-four hour dosage schedule (49). Just what is the significance of this finding is not clear. Also, patients with acute septic infections of the skin and subcutaneous tissues, mainly staphylococcal, responded as well to aqueous penicillin twice daily as to the injection of procaine penicillin (44). Other authors (98) state that discontinuous penicillin provides entirely satisfactory treatment for pneumonia and the great majority of penicillin-susceptible infections. However, they believe that such discontinuous therapy will be inadequate in the treatment of serious staphylococcal infections, bacterial endocarditis, and certain other rarely encountered infections which require large amounts of penicillin. No proof for this belief in the inadequacy of discontinuous therapy is given.

A different point of view in regard to the dosage schedule of penicillin has been proposed in a long series of papers by Eagle and coworkers. At first, Eagle appeared to accept completely the current idea that it is necessary to maintain, day and night, an effective concentration of penicillin in the plasma for effective therapy. In addition, he believed that *in vitro* results on the sensitivity of parasites to the drug could be transferred without modification to infections in man, as the chemotherapeutic effect of penicillin was a bactericidal one (27). However, evidence had already been given that the body defense mechanisms play a role in the cure of infections with penicillin. McLeod and Stone (52) produced infections in mice with types I and III pneumococcus, which had the same virulence for mice and the same sensitivity to penicillin *in vitro*. Infection with type I was much more easily cured by penicillin than infection with type III. However, Eagle, although he has modified his point of view, still appears to maintain that most effective therapy can be obtained by maintaining a continuous bactericidal

concentration of penicillin in plasma. We can quote several statements from his numerous papers to show this. In 1948, he writes "It is a reasonable surmise that the concentration of penicillin which is maximally effective in vitro indicates the approximate level which should be maintained at the focus of infection in vivo in order to kill the largest number of organisms in the shortest possible time" (27). In 1950, he writes "A schedule of treatment which provides the maximally effective bactericidal concentration at the focus of infection continuously should therefore be more rapidly effective than a discontinuous schedule" (29). And again, after accepting the idea that the host defense mechanisms do play a role in the action of penicillin and that, as shown by Parker and Marsh (72), and Parker and Luse (71), bacteria exposed to penicillin *in vitro* did not immediately resume multiplication when the drug was removed, he writes, "Although penicillin can therefore be administered on an intermittent schedule without necessarily maintaining its concentration at an effective level, it is a reasonable presumption that bacteria would die more rapidly under the combined impact of penicillin and the host defenses than under the latter alone, and that a maintained bactericidal level would therefore be more rapidly effective than a schedule of treatment supplying that bactericidal concentration only intermittently" (28). In other papers are given tables to show how often a given dose of penicillin must be repeated or what dose must be administered at fixed intervals in order to maintain the concentration of drug in plasma above a certain level (27, 100).

Eagle brings into consideration the fact that the concentration of drug in the plasma does not represent the concentration at the focus of infection. He states, "Due consideration must be given to the fact that most of the bacteria are usually not in the blood stream but in the tissues, and that the plasma level is not necessarily a measure of the actual concentration at the focus of infection" (26). Then another concept is introduced. In discussing the discrepancies of different workers as to whether or not a maintained concentration of penicillin in plasma is necessary for most effective therapy, he writes, "Additional data will be presented herein which are believed to reconcile some of the apparent discrepancies and to reinforce the thesis that the major determinant factor in the therapeutic activity in penicillin is the aggregate time, not necessarily continuous, for which the drug remains at bactericidal levels" (29).

In regard to the first of these concepts that the bacteria are not in the blood stream but in the tissues and that it is the concentration of penicillin in the tissues which is important, the following may be noted. In bacterial infections, the bacteria are not in tissues but in extracellular fluid with the exception of those present in the leucocytes and macrophages. It is, therefore, the concentration of penicillin in extracellular fluid and not that in tissue cells which is important in combating infection. It is also certain that the concentration of free penicillin (that not bound to the plasma albumin) which is the effective agent is roughly the same in plasma and extracellular fluid after equilibrium has been attained. Equilibrium is probably attained or nearly attained very rapidly. After an intravenous injection there is an abrupt fall in the concentration in the plasma for the first ten minutes or so, due presumably to mixing and diffusion into extracellular

space. After this there is a sharp change in the slope of the curve (40). Richardson *et al.* (78) found the apparent volume of distribution of penicillin to be 20 per cent, that is, mainly distributed in extracellular fluid. Determinations of the concentrations of penicillin in plasma and in extracellular fluids (edema, synovial, ascitic and pericardial fluids) are consistent with the concentration of free penicillin being the same as that in plasma, but the inadequacy of analytical methods for estimating the drug prevents drawing definite conclusions.

The above considerations in regard to the penetration of penicillin into extracellular fluids and the rapidity of the attainment of equilibrium may not apply in certain instances. The slow passage of this drug across the "blood-brain barrier" into the cerebrospinal fluid and the possibly slow attainment of equilibrium in normally avascular areas (*e.g.*, vegetations on heart valves) or areas rendered avascular by disease processes (*e.g.*, lung in Friedländer's pneumonia) may be mentioned. Here, it would appear that high peak concentrations in plasma as given by intermittent as opposed to continuous therapy might increase the rate of penetration of penicillin to such regions and hence be more effective. In addition, it is well recognized that cultures of bacteria are composed of both actively growing and resting organisms. On the basis of this observation and the knowledge that penicillin is most effective against rapidly growing organisms, Bigger (5) has maintained that discontinuous administration of penicillin should be more effective than continuous treatment.

The evidence that the concentration of penicillin varies widely in different organs and tissues from the concentration in the plasma (75, 92, 70, 17, 19) is unsatisfactory. As stated above, the microbiological method is full of pitfalls and when applied to tissues is probably much less reliable than when used with plasma or serum. Indeed, in the only study in which I can find recoveries done with tissues, these were as low as 35 per cent, and no data are given to indicate variability (78). No critical analytical chemist would think of trusting data obtained by such a method. The tissue distribution of penicillin must await the devising of an accurate, specific chemical method for its estimation.

The second concept, that the therapeutic activity of penicillin is proportional to the total time, not necessarily continuous, that an effective concentration is maintained, would imply that continuous administration of the drug is more effective in the sense of producing more rapid cure and requiring less total drug. This is directly opposed to all the evidence obtained with varying dosage schedules in infections in experimental animals. Omitting consideration of the large possible errors in the figures used for the calculation of the "total penicillin time," the "relative constancy of this time factor in a given infection" is found actually to have a variation of at least four-fold.

In experimental infections of rabbits with syphilis, studies on dosage schedules were all carried out very early with crude penicillin of varying composition. In one study, it was found that the length of treatment appeared more important to effect a cure than the dosage of the drug. A large single injection was not effective and sixteen injections at hourly intervals were much less effective than thirty-two injections given twice daily at eight-hour intervals (14). The apparent con-

clusions of a second study (31) are as follows: where the interval between injection was fixed and the number of injections varied, the smallest total dose of penicillin necessary to cure was that associated with the largest number of injections or the maximal duration of treatment; where the duration of treatment was constant and the interval between injections varied, it appears that the greater the number of injections, the greater the efficacy of penicillin; where the total number of injections was held constant, the curative dose depended on the interval between injections, doses at intervals of 1 to 2 hours being less effective than doses given twice daily or daily. In a third study (32), "experiments were undertaken to establish the fact that a single injection of penicillin in oil and beeswax, resulting in a prolonged low blood level, was more effective therapeutically than a single injection of the aqueous solution, and just as effective as multiple small injections. . . ." It was found that when injections were made daily for four days, penicillin in oil and beeswax was more effective than aqueous penicillin, and that two daily injections of penicillin in oil and beeswax for 8 days was more effective than a single injection or four daily injections.

In all three of the above studies amorphous crude penicillin of unknown composition (and of varying activity per unit in rabbit syphilis) was used.[2] In the first study, preparations prepared by six different manufacturers were used; in the second, eighteen different lots from nine different sources, which were used, varied in activity from 130 to 1025 units per milligram; and in the third, nine different lots, four from commercial sources and the rest homemade, were employed. The very small number of animals used makes it impossible to detect differences in activity between the different lots of penicillin. In addition, all these studies attempted to decide too many issues with too few animals; there is a lack of any attempt to estimate the standard errors, which in many cases cannot even be calculated. Therefore, one must accept with reservations any conclusions on the most effective dosage schedule in rabbit syphilis.

There are no data which led to unequivocal conclusions as to the most efficient method of using penicillin in the treatment of human syphilis. It has been assumed that the best method of treatment is one which attempts to maintain a more or less constant and effective concentration of penicillin in the plasma. Due to this preconception about therapy, little is known about the curative effect of widely spaced doses of aqueous penicillin. The conduct of satisfactory, well-controlled experiments in the treatment of human syphilis is beset with many difficulties. Among these are the difficulty of distinguishing relapse and reinfection, the long period of follow-up necessary with its inevitable loss of patients from the study, and the criteria of failure used.

The reports of the Central Statistical Unit in a Nation-wide Study of the

[2] The potency of penicillin in units is determined by its *in vitro* activity against a strain of *S. aureus*. Different species (G, F, X and K) show different activities; G possesses a potency of 1667 units per milligram; F, of 1550 units; X, of 900 units; K, of 2300 units. Compared to G, penicillins F, X, and K have much less activity in rabbit syphilis (3, 102). For further discussion of amorphous penicillin and its changing character see the Report of Committee on Medical Research, The United States Public Health Service and the Food and Drug Administration (16).

Treatment of Early Syphilis (67, 66, 79) give some information on the efficacy of various dosage schedules of penicillin. Cumulative failure rates were used as a criterion of efficacy. In the early studies with amorphous crude penicillin, no significant differences in failure rates could be detected when any total dose from 1.2 to 4.8 million units was given within four days to two weeks at intervals of 2 to 6 hours. In a later more satisfactorily planned study, it was found that using crystalline penicillin, no differences were found with four schedules involving a total dosage of 2.4 and 4.8 million units for 7.5 days and intervals of 2 and 3 hours between doses (65). It is difficult to understand the finding that crystalline penicillin G given in total dosage of 2.4 and 4.8 million units over 7.5 days gives results superior to comparable dosage schedules of amorphous crude penicillin (79). In view of the fact that no difference was found in treatment using a total dosage of 1.2, 2.4, and 4.8 million units of amorphous penicillin, it would appear that the unitage of the amorphous penicillin used at this time did not at all correlate with its antisyphilitic activity.

In regard to the effect of varying the duration of treatment, the Central Statistical Unit found no difference in regimes of 4, 7.5 and 15 days. In addition, it has been found that very large doses of penicillin given over a twenty-four hour period are grossly inadequate for the treatment of syphilis (73).

Due to practical considerations, the various types of depositary penicillin have almost replaced aqueous penicillin in the treatment of syphilis. The desire is apparently to use the one which gives a measurable concentration of antibacterial substance in the plasma for the longest time; at present, procaine penicillin in oil with aluminum monostearate appears to be the most used preparation.

From the above discussion, it appears to the reviewer that it is impossible to state that the most effective dosage schedule of penicillin for the treatment of syphilis requires a maintained concentration in plasma; fairly widely spaced injections of aqueous penicillin may be just as effective.

The situation in regard to the dosage schedule of streptomycin in bacterial infections was quite similar to that with penicillin. Although the concentration of drug in blood is maintained much longer from an injection of streptomycin than from one of penicillin (54), the original dosage schedule of streptomycin required injection of drug every three or four hours. Experiments, however, showed that just as effective therapy is obtained when the drug is given infrequently as when it is given every few hours around the clock. Thus, in a *K. pneumoniae* infection of mice, Zubrod (110) found that the total daily dose of streptomycin administered, rather than the number of fractions into which the total dose is divided, conditioned the therapeutic response. In experimental tuberculosis in the guinea pig, it has been shown 1) that the effectiveness of the drug depends on the length of treatment (91), and 2) that a dose once a day or even at less frequent intervals is just as effective as a dosage schedule requiring administration at four- or six-hour intervals (36, 35).

In human tubercular infection, a dosage schedule of streptomycin which does not maintain a more or less constant concentration of drug in the blood is effec-

tive. Thus, one dose a day (6) and even doses only twice a week (77) are considered effective.

No data are available on the most effective dosage schedule of para-aminosalicylic acid in tuberculosis. It has been given in frequent divided doses mainly in order to give as much drug as possible without disturbing gastrointestinal symptoms.

In regard to the most effective dosage schedules of aureomycin, terramycin and chloramphenicol, no statement of any kind can be made. There is a complete lack of data bearing on the problem. With aureomycin, a limited study of bacterial infections in mice showed that a single large injection of drug immediately after infection was less effective than a dosage schedule of three times daily for three days (11). This, however, has little bearing on the problem of continuous versus intermittent administration. In clinical use, all three of these drugs are usually administered every four or six hours, possibly the best spacing to maintain effective concentrations in blood and to prevent or minimize gastrointestinal irritation.

Although Moore and Chesney were concerned with the need of the most satisfactory dosage schedule of ethylhydrocuprein over a third of a century ago, today little attention is given in the clinical use of new chemotherapeutic agents to finding the most effective and satisfactory method of administration. In the case of the sulfonamides, the dosage schedule used by Moore and Chesney for ethylhydrocuprein was shown to be the most effective and therapy was actually based upon concentrations of drug in blood rather than dosage by mouth. In the case of the antimalarial drugs, the dosage schedules, while to some extent a carry over from the sulfonamides, are probably correct. With penicillin, there is little relation between the clinical dosage schedule in use and the facts obtained from experimental therapy. With the newer antibiotics even experiments on dosage schedules in infected animals are lacking.

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