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AN OVERVIEW OF THE MAJOR ANTIBIOTICS IN EQUINE MEDICINE†

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THE PENICILLINS

The penicillins kill bacteria by robbing them of their cell walls. As illustrated in Figure 1, the normal bacterium lives inside its tough cell wall which protects it from osmotic rupture. The last step in the formation of this cell wall is the opening of an alanine-alanine bond and its "cross" linking with special groups on an adjacent chain to complete the cell wall. The action of penicillin is to specifically block the enzyme which splits the alanine-alanine bond and in this way, block the final step in cell wall formation (Fig. 1).[1]

Penicillin blocks the enzyme which splits the alanine-alanine bond by "fooling" the cross linking enzyme. It turns out that a portion of the penicillin molecule (technically called the β lactam bond) looks just like the alanine-alanine bond (Fig. 2). The cross linking enzyme binds the penicillin molecule in just the say way as it would bind the alanine-alanine bond, and then tries to open the β lactam bond of the penicillin molecule. The cross linking enzyme progresses about half-way and then cannot go any further. The process stops with the penicillin covalently and irreversibly bound to the active site of the cross linking enzyme. The cross linking enzyme has in fact "bitten off" more than it can chew, and winds up with a penicillin molecule "stuck in its throat"! Since the penicillin binds irreversibly to the cross linking enzyme, the enzyme can no longer make bacterial cell wall. Without the protection of the tough bacterial cell wall, the bacteria swell up, burst and die (Fig. 1).

Many of the clinical actions and effects of penicillin in the horse relate directly to this mechanism of action. Penicillin is categorized as a bactericidal drug which means that it actually causes bacterial death, rather than merely stopping growth of bacteria. When penicillin is added to a culture of rapidly growing bacteria, it immediately stops further growth and then causes death of all bacteria in the culture (or the horse). On the other hand, if an antibiotic which was simply bacteriostatic was used, further growth of the bacteria would stop but bacterial death would not occur. Obviously, in a clinical situation, a bactericidal drug is preferable to a bacteriostatic one, and penicillin is one of the most potent bactericidal agents known.

Because penicillin only blocks formation of new cell wall, it is primarily effective against "young," rapidly growing populations of bacteria. If the bacteria are "mature" and not forming new cell wall, penicillin is much less effective. This is also the reason why bacteriostatic drugs antagonize the actions of penicillin, because they simply prevent bacterial growth and thus the formation of new cell wall, thereby blocking the action of penicillin.[1]

The very tight or "irreversible" binding of penicillin to the cross linking enzyme is the principal reason for the potency and effectiveness of penicillin.

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Unlike penicillin, most other antibiotics, and indeed most drugs, only bind to their receptors for a few seconds and then diffuse away from the receptor. With penicillin, however, the irreversible binding means that once the penicillin has linked to the cross linking enzyme, it stays there. Because of this, only relatively little penicillin is required to block the cross linking enzymes, thus penicillin is a very potent drug. The irreversible nature of the binding also means that penicillin is one of the few drugs the blood levels of which can be allowed to drop, since the drug will remain bound to the receptors. This practice is, however, not to be encouraged.

Another clinical characteristic of penicillin which relates to its mechanism of action is that it may be ineffective in certain areas of the body. Because penicillin does not kill bacteria directly, but merely robs them of their cell walls, it is possible for bacteria to "persist" without their cell walls in certain areas of the body. These areas are usually areas of high osmotic pressure such as the kidney and, possibly, certain abscesses. Bacteria may thus "persist" in these protected areas during therapy and then "re-infect" the animal when the drug is withdrawn. In the fact of such "persisters", another type of antibiotic needs to be selected for a good therapeutic effect.'

Potent and specific as penicillin is, however, bacteria are far from helpless against it, and one of the most common methods by which they fight back is penicillinase. Penicillinase is an enzyme which splits penicillin, usually at the β-lactam bond, and nowadays bacteria which produce penicillinase have a considerable advantage and are becoming increasingly common. Their appearance can be a clinical problem and has in turn led drug companies to develop penicillins resistant to the actions of these enzymes. These are the penicillinase-resistant penicillins which are now an important part of the veterinarian's spectrum of drugs.

Among all the penicillins, penicillin G or benzyl penicillin is the most potent, most effective and cheapest of the penicillins. It is, therefore, all other things being equal, the drug of choice from this group. However, penicillin G has a number of disadvantages and, to get around these disadvantages, the semi-synthetic penicillins were developed.

The semi-synthetic penicillins were developed to deal with the problems that one can run into with penicillin G. Penicillin G is broken down in the presence of stomach acid, so acid-resistant forms of penicillin were developed. Penicillin G can also be inactivated by penicillinase-producing organisms, so the penicillinase-resistant organisms were developed. Lastly, penicillin G is almost inactive against Gram-negative organisms, so the broad spectrum penicillins were developed which are active against both Gram-postive and Gram-negative bacteria.

In performing these modifications, the first step was to grow the basic penicillin nucleus in culture, different synthetic chemical groups being added to the left hand portion of the molecule as shown in Figure 2. Because half the molecule is grown in a culture of the penicillium mold, and the other half is synthetic, they are called the semi-synthetic penicillins. Because these changes are modifications of the potent penicillin G molecule, the semi-synthetic penicillins are all less potent than penicillin G. It is a tribute to the great potency of the penicillin molecule that it can be manipulated so much and still retain useful antibacterial activity.

Having selected the type of penicillins to administer, the next problem that the veterinarian faces is how to administer it. Penicillin G, the penicillin most likely to be administered, is available in three different dosage forms, as the water-soluble sodium or potassium salts, as procaine penicillin and, less commonly, as benzathine penicillin.

Since the sodium and potassium salts of penicillin are water-soluble, they may be given intravenously or intramuscularly. When given by either route, a

very high blood level of penicillin is achieved initially, but falls away rapidly over the next 12 hours or so. This rapid fall in the blood level of penicillin occurs because as an organic acid, penicillin is pumped directly into the urine in much the same way as furosemide is. This rapid decrease in the blood levels of penicillin is a therapeutic problem, since it means that adequate blood levels of penicillin are likely to be difficult to maintain (Fig. 3).

One solution to this problem of the rapid elimination of penicillin is to inject the drug intramuscularly in a form in which it is only slowly absorbed. When given this way, penicillin trickles into the bloodstream from its i.m. injection site and, as long as it continues to do so, an adequate blood level of penicillin is obtained. The problem with this approach, however, is that very high blood levels of penicillin are not achieved.

Procaine penicillin is the most commonly used depot or slowly-absorbed form of penicillin and, with this preparation, penicillin levels in the bloodstream of above 2μg./ml. are rarely reached. This is a marginal concentration of penicillin to have in the bloodstream if the organism is in any way resistant to penicillin. In addition, penicillin is not a particularly good penetrator into tissues, but the high initial blood levels obtained after injection of Na + and K + penicillin will yield higher tissue levels of drug than may be expected after procaine penicillin. Therefore, if an infection is located in a protected site such as a joint, abscess or in the central nervous system, it may be better to give frequent large doses of Na + or K + penicillin rather than equivalent doses of procaine penicillin (Fig. 3). Finally, because procaine penicillin is an insoluble form of the drug, it should never be given intravenously, as administration by this route may cause massive pulmonary embolism and death.[7]

Methacillin is one of the principal penicillinase-resistant penicillins. This drug has been modified so that when it is attacked by the penicillinase enzyme it binds to and inactivates it. It is, however, much less potent than penicillin G and higher levels of it are required to produce the same antibacterial effect. On the other hand, since methacillin acts to block penicillinase, there is no reason that one cannot combine penicillin G, which is cheap, with methacillin, which is more expensive, but which will block any penicillinase present and allow penicillin G to act on the bacteria.

Until the relatively recent development of the broad spectrum penicillins, the penicillins were essentially inactive against Gram-negative organisms. With the advent of ampicillin, however, the first of the broad spectrum penicillins was introduced into medicine. While ampicillin is only half as potent as penicillin G against Gram-positive organisms, it is up to 10 times more potent than penicillin G against various Gram-negative organisms. One point to remember about ampicillin, however, is that the ampicillin sodium gives much better blood levels of ampicillin than ampicillin trihydrate and is thus likely to give better clinical results.

Amoxicillin is a recently introduced broad spectrum penicillin which seems to be more bio-available than the other broad spectrum penicillins. When administered systemically, higher blood levels may be obtained than with the other broad spectrum penicillins. As well as giving better blood levels, it appears to penetrate bacteria more rapidly and thus lead to more rapid bacterial death than with other broad spectrum agents.

As pointed out earlier, penicillin is essentially non-toxic in the horse. About the only direct toxicity associated with penicillin is its ability to cause convulsions if given in very high doses, and to my knowledge, this problem has never been reported in the horse. A much more likely problem is cation toxicity, for if large doses of potassium penicillin are given i.v., the added potassium ion may depress the heart. All other things being equal, therefore, the sodium salt is the penicillin of choice for intravenous use. Occasionally, when large doses of procaine penicillin are given, horses may go through an acute excitement

response. A similar sequence is occasionally seen in man, and its cause remains unclear. Finally, as with any drug, allergy or hypersensitivity problems may be seen. The incidence of this is higher with the penicillins than with other drugs. Finally, the major problem with the penicillins in performance horses is the ability of the procaine in procaine penicillin to turn up in horse urine for up to two weeks after a dose of penicillin procaine, giving rise to "inadvertent" positives due to procaine (Fig. 4).*

When it comes to using the penicillins in an individual case, it should be remembered that penicillin G is the least expensive and most potent of all the penicillins. Further, because the penicillins are essentially non-toxic, there is no reason to restrict the dose since it is difficult to overdose with the penicillins. Currently, doses of procaine penicillin administered intramuscularly to human beings range up to 5 million units, which must therefore be considered a very conservative dose for a horse. It is also important to remember than high plasma levels of drug aid the diffusion of penicillin into infected areas and in an acute situation my own preference would be for frequent high doses of sodium penicillin rather than less frequent doses of procaine penicillin.

If the microorganism causing the problem is penicillinase-producing, methicillin or amoxicillin may be used. Full dosage schedules of these agents should be used and it is probably advantageous to combine these drugs with penicillin G since these drugs act by blocking penicillinase and will thereby protect the more potent penicillin G from hydrolysis and allow it to act.

Among the broad spectrum agents, ampicillin or amoxicillin appear to be the drugs of choice, with amoxicillin appearing to give better blood levels and more rapid action. Carbenicillin is the agent of choice in treating Pseudomonas infections, usually in combination with gentamicin, the most effective antibiotic of the aminoglycoside group.

GENTAMICIN AND THE AMINOGLYCOSIDES

Gentamicin is currently the most important member of the aminoglycoside family of antibiotics, of which streptomycin was the first member discovered. The discovery of streptomycin in 1944 was followed by neomycin (1949), kanamycin (1957), gentamicin (1963) and others. As a group, these agents are known as the aminoglycoside antibiotics, and they share in common a large number of pharmacological and therapeutic properties.

The characteristic which completely dominates the pharmacology of this family of antibiotics is their high water-solubility and low lipid-solubility. Even casual inspection of the structure of gentamicin (Fig. 5) shows it to contain many OH (hydroxyl) and NH groups. These groups interact well with water and the net result is that streptomycin, gentamicin and the other members of this family cross the lipid outer membrane of cells with difficulty. They are therefore all poorly absorbed after oral administration and are distributed poorly in the body even if given by injection. They cross into the brain with extreme difficulty, enter the eye poorly and even have trouble getting into red cells. Because they do not enter liver cells, they are not metabolized to any significant extent and are not excreted in the bile. They are therefore excreted largely unchanged by the kidneys, and since they are concentrated by the renal concentrating mechanisms, they are found in high concentrations in urine. Because of the many characteristics that this group of drugs have in common, the pharmacology of gentamicin will be presented first and then compared with the others of the group.

Like all members of this group, gentamicin is poorly absorbed from the intestinal tract and acts essentially only on the intestinal tract after oral administration. For systemic action, gentamicin must be given parenterally, usually by intramuscular injection. Although well absorbed after i.m. injection, gentamicin is not distributed particularly well in the body and enters the brain

and the eye only with difficulty. Good levels which compare well with those in the blood are found in the peritoneal fluid but levels in joint cavities and synovial fluid tend to be much lower.

As a highly water-soluble molecule, gentamicin is excreted through the kidney and has little or no tendency to be absorbed from the renal tubules. Its plasma half-life is therefore in the order of about 2-3 hours, as is the case for all the antibiotics of this group. They must therefore be given at frequent intervals to maintain effective blood levels.

Blood levels of about 4 μg./ml. are usually considered to be the minimum desirable levels in the horse, and Fig. 6 shows that dosing every six hours is required to approach this goal. When gentamicin is given at a rate of 3.3 mg./kg. i.m., peak blood levels of about 8 μg./ml. are obtained, dropping to about 2 μg./ml. at six hours. If the dose is increased to 4.4 mg./lb., the peak blood level is about 15 μg./ml., dropping to about 3 μg./ml. after six hours. Dosing every six hours with gentamicin is required, therefore, to maintain minimal blood levels of this drug.[1]

Gentamicin and the other members of this group are generally considered to be bactericidal drugs, although at low drug concentrations or in the presence of relatively resistant organisms, they may be only bacteriostatic. As far as is known, all the members of this group produce their bactericidal effect by binding specifically, and apparently reversibly, to a part of the protein synthesis machinery of the bacterium called the 30S ribosomal sub-unit. In susceptible bacteria these drugs lead to the synthesis by the bacterium of what are called "silent" or "non-sense" proteins, *i.e.* "enzymes" without catalytic activity, or "structural proteins" which do not fit anywhere in the bacterium. This gentamicin-induced inability of the bacterium to synthesize the functional proteins it requires, leads to death of the bacterium and accounts for the bactericidal action of gentamicin and the other members of this group.[4]

All the aminoglycoside antibiotics are active principally against Gram-negative organisms and gentamicin is the drug of first choice when a severe infection due to one of these agents is suspected. Because all the agents of this group are excreted unchanged in the urine, they attain high concentration there and can be very effective in urinary tract infections. Further, if the urine is alkaline, the antibacterial activity of these agents may be substantially increased. As mentioned earlier, the treatment of choice for Pseudomonas infections which can be very difficult to treat, is a combination of carbenicillin and gentamicin. When using this combination, however, it should be remembered that these agents are chemically incompatible so they should not be mixed together in solution, as they will inactivate each other.[1]

Streptomycin is now rarely used alone in equine medicine, but almost always as part of a penicillin-streptomycin combination. The principal problem with streptomycin is that resistance to it is both relatively common and occurs readily, thus greatly reducing its usefulness. Like the other members of this group, streptomycin is relatively rapidly excreted, and should be given in three daily doses at about 20 mg./kg. Because of its rapid excretion there is little danger of cumulation of this drug if renal function is normal but if renal function is impaired, high blood levels of streptomycin and toxicity can readily result.

Kanamycin is the second most effective drug in this group, but is less active and more toxic than gentamicin. Its plasma half-life is relatively short, with an apparent half-life of about 1.5 hours, which makes it difficult to maintain blood levels. Knight in California, suggests a dosage rate of about 5 mg./kg., three times daily.[1]

Neomycin is closely related to kanamycin but is even more toxic, which limits its usefulness. In human medicine it is rarely used systemically because of its toxicity. Neomycin is principally used, therefore, as a topical preparation for

infections of the eyes, ears and skin, and to "sterilize" the bowel prior to surgery. Neomycin has been suggested for systemic use in the horse along with ampicillin in the treatment of contagious equine metritis, but is role and efficacy in such therapy remains to be determined.

Spectinomycin is an aminocyclitol antibiotic rather than an aminoglycoside, and thus its actions and toxicity differ substantially from those of a typical aminoglycoside. It is less active on a molar basis than this group of drugs and data from man suggest dosage rates of up to 40 mg./kg. parenterally. It has been used in the horse with good results as an intrauterine infusion in the treatment of mixed uterine infections and infections due to Klebsiella. When administered in this way, the dose for an adult horse is about 2 to 3 Gm. of spectinomycin in a 500 ml. volume, daily for three to four days. Because spectinomycin is not an aminoglycoside, it can be used in high doses without fear of inducing eighth nerve damage or renal toxicity. Adverse reactions reported in man include dizziness, vertigo, malaise and anorexia. Spectinomycin may also be used parenterally in the horse and dosage schedules and indications for its use by this route in the horse are now being worked out.

In summary, gentamicin is clearly the aminoglycoside of choice in the therapy of most Gram-negative infections in horses and, in combination with carbenicillin, is especially useful in treating deep-seated infections due to *Pseudomonas spp.* Gentamicin should, however, be used with care in young foals, and all horses on gentamicin should be watched carefully for signs of renal damage. Gentamicin has essentially replaced kanamycin in equine medicine and relegated streptomycin to use in penicillin-streptomycin combinations. While a number of new aminoglycoside antibiotics have recently been developed, these, with the possible exception of amikacin and spectinomycin, have at present no readily apparent advantages over gentamicin.

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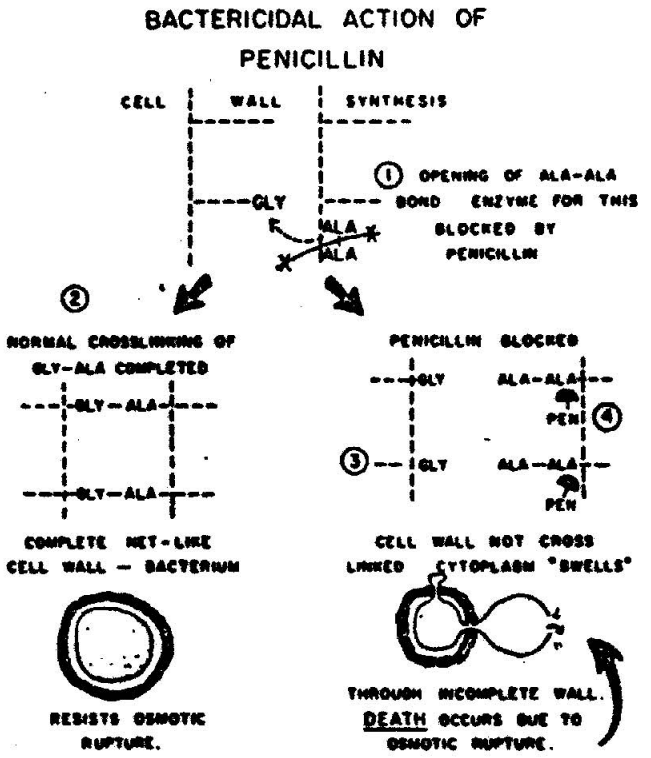

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Fig. 1: Bactericidal Action of Penicillin.

The last step in bacterial cell wall synthesis is the opening of an alanine-alanine bond at (1) and the linking of the remaining alanine to a glycine on the next strand. This cross-linking (2) completes the cell wall which protects the bacterium from osmotic rupture. In the presence of penicillin, the cross-linking enzyme attacks and attempts to open the β-lactam bond of the penicillin molecule. Penicillin binds covalently to the active site on the enzyme (Pen—E), blocking its action with the result that the cell wall is not cross-linked and the bacterium is not protected against osmotic shock. Bacterial death, when it occurs, is due to osmotic rupture and not to any direct action of penicillin.

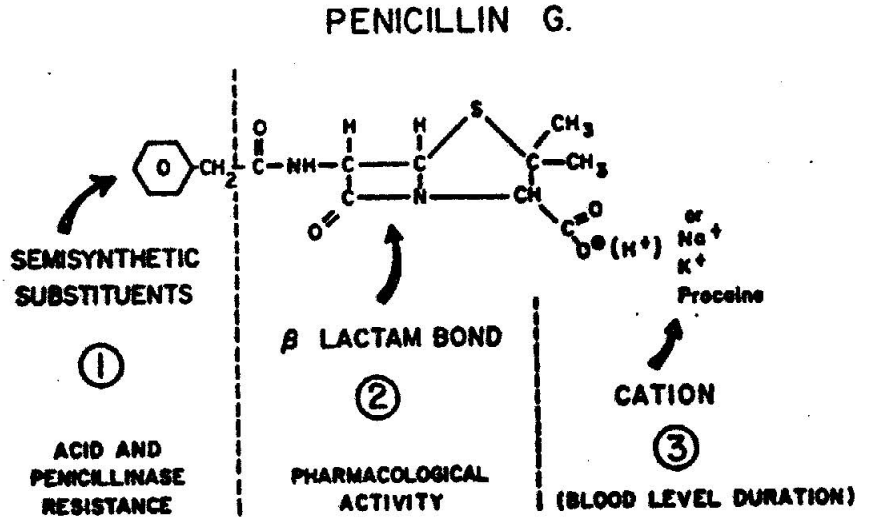

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Fig. 2: Structure and Activity of Penicillin G.

The antibacterial activity of penicillin resides in the CN β-lactam bond (2) which is attacked by, but blocks, the bacterial "cross-linking" enzyme. This lactam bond is sensitive to acidic or penicillinase hydrolysis. Alterations at (1) on the penicillin molecule give rise to the semi-synthetic penicillins which can be acid stable (oxacillin), penicillinase-resistant (methicillin) or broad spectrum (ampicillin). In crystalline penicillins, the H at (3) is substituted for by Na+ or K+, which gives rise to highly water soluble compounds suitable for intravenous administration. If procaine is substituted for these ions, a slowly dissolving crystal is formed which is a useful intramuscular depot preparation of penicillin.

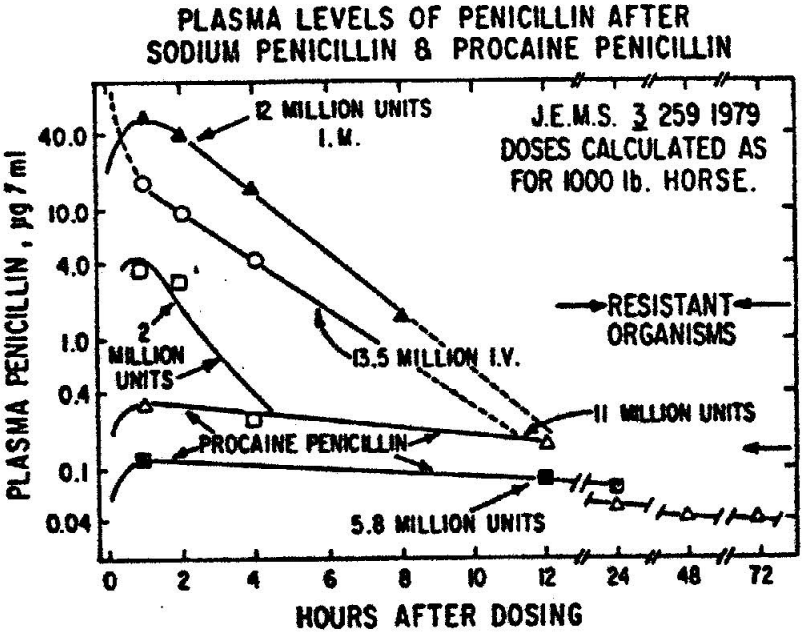

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Fig. 3: Plasma Levels of Penicillin after Sodium Penicillin i.v. or i.m. and Procaine Penicillin.

The data show the different rates of absorption and elimination of penicillin when administered in different forms. The open circles (o-o) show plasma levels of penicillin after about 13.5 million units of sodium penicillin i.v., while the solid triangles (▲-▲) show plasma levels after about the same dose was given i.m. In both cases, peak plasma levels of the drug of about 40 µg./ml. were obtained but which decayed rapidly. If the drug was given intramuscularly as procaine penicillin, however, 100-fold lower blood levels of drug were obtained initially but these lower levels declined much more slowly and small blood levels of penicillin were still observed 72 hours after dosing.

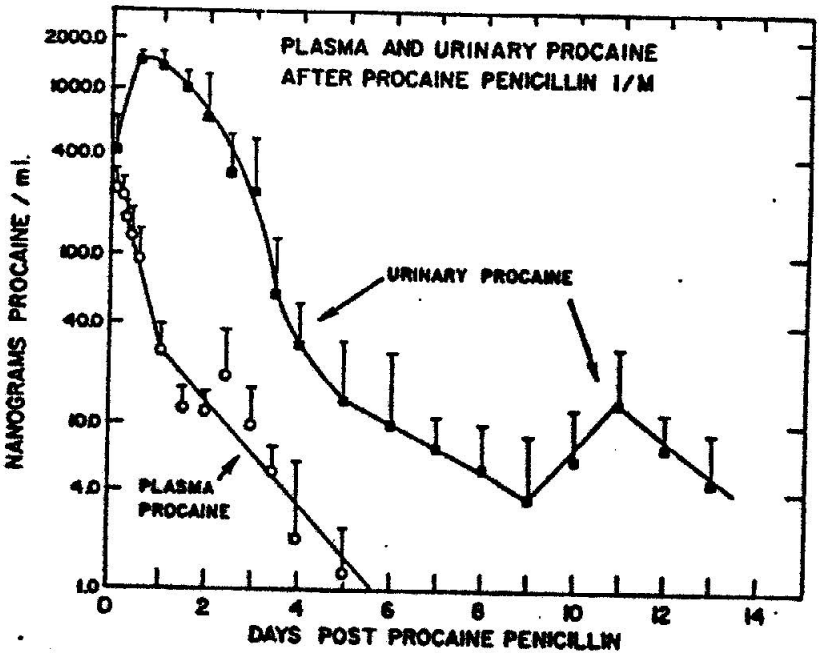

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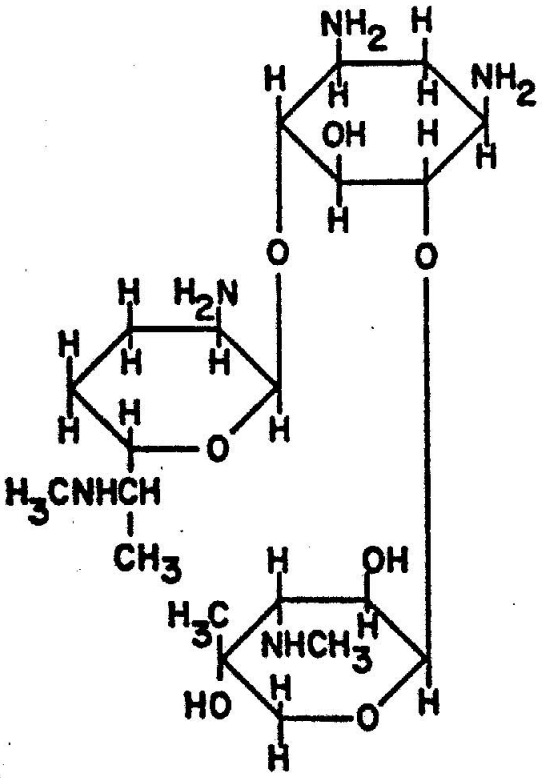
Fig. 4: Plasma and Urinary Concentrations of Procaine after Intramuscular Administration of Procaine Penicillin.

The open circles (o-o) show plasma concentrations of procaine after the intramuscular administration of 33,000 I.U./kg. of procaine penicillin to Thoroughbred horses. The solid squares (▪-▪) show urinary concentrations of procaine in these experiments. All data points are means ± of experiments on four different horses.



GENTAMICIN

Courtesy of *The Journal of Equine Medicine and Surgery.*

Fig. 5: Structure of Gentamicin.

The large number of OH and NH groups on the gentamicin molecule render it highly water-soluble and poorly lipid-soluble.

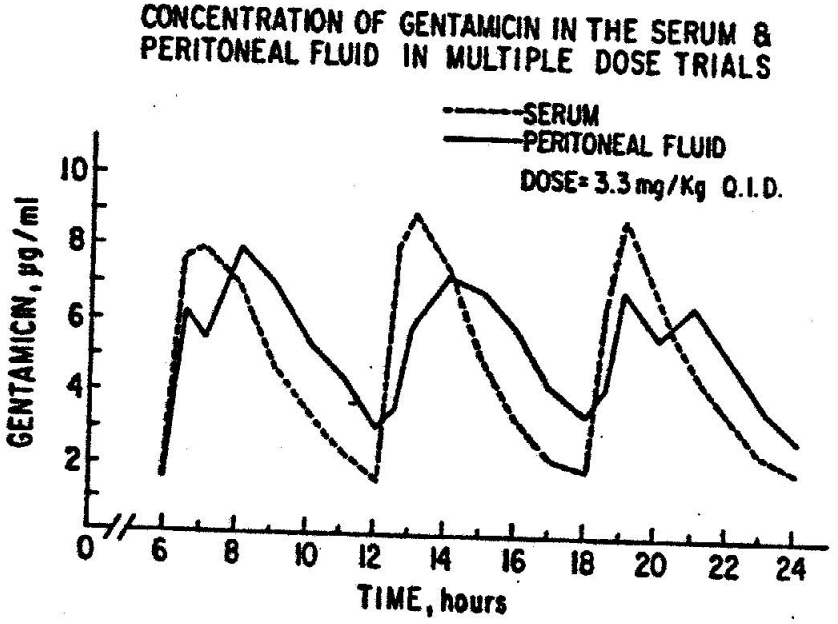


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Fig. 6: Concentration of Gentamicin in the Serum and Peritoneal Fluid in Multiple Dose Trials.

The dashed line (----) shows serum levels of gentamicin after dosing with 3.3 mg./kg. of gentamicin every six hours, while the solid lines show peritoneal fluid levels.