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Penicillin

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FLEMMING'S accidental discovery of penicillin in 1929 brought about the problem of extracting this antibacterial substance in a form purified enough to be tested clinically. The solving of this problem was undertaken by many research workers, and under the leadership of Chain and Florey (3) a group of Oxford workers revealed, in August of 1940, that they had devised methods for obtaining a considerable yield of penicillin and for making a rapid assay of its inhibitory power. After considerable experimentation with the culture medium, a brown powder was finally obtained which was freely soluble in water. The antibacterial power of this comparatively stable powder and solution proved to be remarkable. In dilutions as great as one in several hundred thousand, the penicillin solution inhibited, by interfering with multiplication, the growth of many different microorganisms. During the course of their therapeutic tests on mice it was found that streptococci, staphylococci, and Clostridium septicum were particularly susceptible to inhibition. The agent also showed a definite activity against the anaerobic organisms associated with gas gangrene.

Again in 1941 this same group set forth a new and detailed description of a large-scale method of producing penicillin. During the course of their studies they gained new knowledge concerning the low toxicity of purified preparations of this agent when applied to body tissues; furthermore, they proved that blood, pus, and tissue breakdown products did not prevent its inhibitory action. Included in this series of observations was the action of sulfonamide drugs as compared to penicillin and it was demonstrated that in heavily infected wounds the activity of penicillin was influenced only to a minor extent by the bacteria present while the sulfa drugs seemed to have little beneficial action. During this time a number of cases of fulminating pyogenic infections in man were being treated by the drug and although the clinical results in most instances were favorable it was found that, due to a lack of the drug itself, many treatments had to be discontinued before a complete cure was achieved. Needless to say, the discovery of penicillin has actually proven to be the greatest advancement in the field of chemotherapy since the advent of the sulfonamides. Moreover, the activity of penicillin remains undiminished under many clinical tests which have completely nullified the effect of the sulfonamides, and in this respect at least the new agent appears incomparably superior to the latter group of compounds. Although the full clinical potentialities of penicillin are yet to be realized, it has at this time reached the stage at which its efficacy in a wide variety of ailments has definitely been established.

Terminology

One of the purposes of this article is to give the reader a more thorough understanding of some of the properties and uses of penicillin. However, before proceeding further it might be wise to devote a few paragraphs to the terminology used
in connection with this antibacterial substance and then give a few of its physical and chemical characteristics.

It can be easily understood why a unit of measurement was necessary for penicillin and due to the lack of chemical methods of determination, penicillin potency was expressed in terms of its antibacterial activity. It was decided to use a purely arbitrary unit, originally defined by the Oxford workers (1), and which at this time has been universally adopted as the Oxford or Florey unit. The Oxford unit, as defined by its originator, is that amount of penicillin which when dissolved in 1 cc. of water gives the same inhibition by the cup assay method as a stable penicillin solution maintained as a reference standard by these workers. Through various experiments, it has been found that this unit is approximately equal to the smallest amount which when dissolved in 50 cc. of meat broth extract, inhibits completely the growth of the test strain of *Staphylococcus aureus*.

**Properties**

At the present time penicillin preparations being produced and supplied for clinical use have a potency of from 100 to 1,000 Oxford units per mg. The sodium salt of this preparation is a light orangish-brown hygroscopic powder having a slight odor. It is soluble in both water and alcohol but inactivated by the latter. The presence of sodium chloride or glucose in the same solution does not affect its activity and for clinical uses it is frequently administered in solution with these substances. In the powdered state penicillin proves relatively stable to light but it is adversely affected by heat in direct relation to the temperature. If the substance is stored at temperatures below 10°C, it will retain its full activity for a period of several months. When the penicillin is being used in an aqueous solution it is kept frozen when not in use and although it may even be kept at ordinary refrigerator temperatures for several days without a significant loss in potency, it is advisable not to store it longer than 24 hours (17).

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### Susceptibility of Organisms to Penicillin

(Hobby et. al. (14)

**Susceptible Strains**

- Pneumococcus
- Streptococcus hemolyticus
- *Staphylococcus*
- Meningococcus
- Gonococcus
- *Streptococcus viridans*
- *B. subtilis*
- Cl. welchii
- V. septicum
- Cl. histolyticus
- *B. sporogenes*
- *B. oedematiens*
- B. sordelli
- Lactobacillus
- Cryptococcus

**Insensitive Strains**

- H. influenzae
- E. coli
- B. typhosus
- B. dysenteriae
- B. proteus
- B. paratyphosus A.
- B. enteritidis
- B. pyocyaneus
- B. fluorescens
- B. prodigiosus
- Friedlander’s bc.
- *Staphylococcus albus—1 strain*
- *Micrococcus albus—1 strain*
- Monilia albicans
- Monilia krusei
- Monilia cardida

Owing to a definite lack of published material in connection with the treatment of large animal cases with penicillin, it is difficult at this time to give much information that might be used by a veterinarian. However, with the great progress in the commercial production of this substance, more and more work is being carried on along the veterinary line and from all indications the results are correlating favorably with the successful results on certain cases in experimental small animals and in man.

A problem of primary interest which is receiving considerable attention by research workers in the veterinary field is the treatment of bovine mastitis with penicillin. In July, 1944, Kakavas (16) published a preliminary report dealing with
the use of this drug in the treating of this disease. Several different preparations of penicillin were reported as being used. It was observed that the aqueous solution of sodium penicillin produced no unfavorable results to the tissue of the udder, as was not the case when crude penicillin, produced in a corn steeping liquor medium, was used. This aqueous preparation was administered to the udder, through the teat canal, immediately after milking. It was then left in the udder until the following milking period. These treatments were repeated at 24 hour intervals. Following this method of treatment, 27 quarters infected with staphylococci were treated. Approximately 59 per cent of these quarters showed favorable results, the remainder giving no definite response. The success of this treatment was determined by testing milk samples which had been taken at 2 and 4 weeks after treatment had been discontinued. If none of the infectious organisms of mastitis significance appeared, after reasonable time was allowed for organism growth on the blood-agar plates, the treatment was considered as being successful.

Raw penicillin whey-broth filtrate was used in treating udders infected with S. agalactiae. From the favorable observations made it was indicated that this organism was much more sensitive to the action of penicillin than Staphylococcus aureus. It was further observed that the total bacterial count of the milk was decidedly decreased and remained so as long as 24 hours after the single dose of 15,000 units was administered. Of significant importance was the fact that milk had no inhibitory effect on penicillin.

**Local Injections**

A more recent report on the effect of local injections of this drug into the cow's udder for the treatment of staphylococci infection was made by Klein et al in January, 1945 (16). The sodium salt of penicillin was used, which, at the time of preparation, assayed 320 units per milligram. However, due to some difficulties, the study was not conducted for some time after receiving the penicillin and at the time treatments were started the assay value was 190 units per milligram. The single doses administered through the teat canal contained 22,800 units of penicillin. Through the course of this experiment 7 quarters were treated. The time interval between administering the drug was compared with 4 quarters being treated with 8 injections at intervals of 6 hours and 3 quarters receiving 4 injections at intervals of 12 hours.

**Results of Therapy**

Numerous samples of milk were taken from the quarters, the first of which was taken before the treatment and the others at varying intervals after. These milk samples showed that before treatment the staphylococcus was present and after the first treatment and during the course of the others the milk samples were staphylococcus free. However, in samples taken from 3 to 6 days after treatment was discontinued it was observed that in 5 of the 7 cases the infection was once again present. The two cases that were staphylococcus free were two out of the three which had received the highest number of single doses. It is difficult to make any definite assumptions from these results other than the fact that the smaller number of doses that were administered did not produce any lasting results.

**Ornithosis**

Closely correlating with the problem of veterinary therapy is the disease diagnosed in man as ornithosis. A case was reported by Turgasen in December of 1944 of a middle aged man, who was a pigeon fancier, as having this disease (19). It is probable that this man contacted the disease from his pigeons because the virus of ornithosis was identified in the enlarged spleen of one of the pigeons. This case was treated with penicillin for a period of seven and one-half days. The drug was administered by the intramuscular route in divided doses using an aqueous solution of sodium chloride with 10,000 units per cc. and a daily amount of 100,000 units. The treatment was ceased after the patient's temperature was normal for three days.
In February, 1944, Heilman and Herrel (11) published a report concerning the use of penicillin in the treatment of experimental Leptospirosis. This disease is often referred to as Weil's disease and in some cases has been called “spirochetal jaundice.” Guinea-pigs were chosen as the experimental animals and preliminary experiments showed that comparatively small doses of penicillin protected these animals against fatal infections of this disease during the course of the treatments. However, as soon as the treatments were stopped relapses would occasionally occur.

**Final Experiments**

For the final experiments the infected animals were treated with the calcium salt of penicillin suspended in sesame oil and administered subcutaneously. The treatments, which consisted of 800 units per day, were carried on for a period of 7 days. On the fifth day after treatment was stopped a number of the treated animals were killed and examined and none were found to have lesions of leptospirosis. Only one of the controls of this group remained alive and upon examination definite lesions were present. In another group of 32 treated animals which were held over for a different period of time there were 9 which gave evidence of relapse from 3 to 7 days after treatment was stopped. Penicillin was again administered to this group of 9 with the result that the temperature returned to normal. At the end of this experiment all but 3 of this group of 32 were alive. The cause of the death of these 3 animals was thought to be due to the toxic effects of the drug. In the control group for these 32 animals there was a mortality of 91 per cent.

**Results**

It is evident from these data that penicillin protected the animals against several times the lethal dose of the organism. There were some cases, however, in which a febrile relapse did occur but as soon as the penicillin was started again this condition was overcome. The main difficulty experienced in this study was the toxic effect of penicillin on the guinea-pigs. If the problem of toxicity could be overcome and consequently larger doses of the drug could be administered it is evident that the infection would be more easily eradicated. From this experimental work it seems logical to believe that penicillin will be useful in the treatment of this type of disease in man and animals.

Heilman and Herrel (12-13) more recently have reported the use of penicillin for the treatment of experimental infections with *Bacillus anthracis* and *Erysipelothrix rhusiopathiae*. For their study of *B. anthracis* young adult Swiss mice were inoculated subcutaneously with the organism and the sodium and calcium salts of penicillin were administered at the rate of 1,000 units per day.

The first phase of the experiment was conducted with 20 treated mice and 20 controls. The treatments, which were started 16 hours after inoculation, were continued for 12 days. All of the controls died within a few days, but only 9 of the treated animals died. The evening dose was administered at 9 o'clock and the following one not until 9 a.m. and it was thought that this long time interval had an unfavorable effect on the infection.

**Second Phase**

The second phase of this experiment involved the same number of mice but treatment was started 1 hour after inoculation and continued for a period of 12 days. The surviving mice were under observation for a period of 26 days. Here again all of the control mice died while there was no mortality in the treated mice. This definitely showed that the organisms of *B. anthracis* were susceptible to treatment with this drug.

For their study of *E. rhusiopathiae* in vitro tests and also tests with mice were performed. It was observed that penicillin was effective against this organism in both cases. For the in vitro tests organisms were placed in a medium conducive to growth and varying amounts of calcium penicillin were added. Three strains of *E. rhusiopathiae* were examined and in every case it was observed that the organisms were sensitive to the action

(Continued on page 153)
Penicillin

(Continued from page 144)

of penicillin. The intensity of the action varying with the amount of penicillin added.

A series of three tests was conducted with mice infected with this organism. The differences of the tests being the time interval between inoculation and treatment, and the amount of lethal doses of the organism injected into the mice. Eighty mice were infected with *E. rhusiopathiae* which had been isolated from an epidemic in turkeys. Out of this number 40 were treated and 40 were held as controls. The mortality in the controls was 100 per cent while it was only 5 per cent in the treated animals. Here again penicillin is shown to be effective against this organism and it is probable that in the future it will be used in the treatment of this disease in swine and in man.

**Clostridium Welchii**

Of major importance to the veterinarian is the study of treatment of *Clostridium welchii* infection with penicillin. Has (7) recently reported the effectiveness of this drug against the experimental infection in mice and guinea-pigs. The mice were treated at 4 hour intervals with 0.25 ml. of penicillin administered subcutaneously into the sub-inguinal region. The same amount was used in treating the guinea-pigs but the drug was administered into the infected muscle itself. It was observed that when the dosage was increased up to 50 units the percentage of survival increased up to approximately 90 per cent. This percentage of survival remained constant until the dosage exceeded 250 units and then it was greatly decreased. Sulfonamides, zinc peroxide and tyrothricin were used against this same organism to determine the effectiveness of penicillin and it was definitely observed that penicillin was far superior to any other chemotherapeutic agent used. Penicillin also proved to be much more effective in speeding up the repair of the tissues and in minimizing the effects of toxemia. However, the author pointed out that adequate surgical procedures are necessary and also additional therapy.

The two routes that may be followed in administering penicillin are intravenous or intramuscular. The latter method is advised in the usual case. The intravenous route is used when the time element is all important and is reserved for patients suffering from immediate life-endangering infections. Often when this drug is given by intramuscular injection, a burning pain follows for about 30 minutes at the site of the injection. However, after the second day of treatment this pain usually disappears. It has been found that local thrombosis of the vein is caused by intermittent intravenous injections. However, active phlebitis is quite uncommon. If the drug was injected by a constant intravenous route, acute phlebitis was frequent, and chills and fever did occur if injections were continued through the same vein. It was reported that in cases of meningitis and brain abscess intrathecal or intraventricular injections were recommended. Penicillin is being used as a local application to wounds and a single daily application in a concentration of 250 units per cc. is quite sufficient. This method has especially been indicated in order to maintain wound sterility after parenteral administration has been discontinued. Also of importance to note is that the sodium salt has proven to be quite irritating and should not be used in powdered form as a local application.

**Recent Experiment**

Recently an interesting experiment was carried on in which the transmission of penicillin through the human placenta was investigated. This work was carried on by Green and Hobby in 1944 and their efforts were directed toward determining the existence of a placental barrier to this agent. Penicillin, in a normal saline solution, was administered intravenously to normal patients in active labor. One test was carried on where the patients were given 100,000 units each and upon examination significant quantities of penicillin were detected in the maternal and placental cord blood. Other cases in which the patients received 20,000 units of the
drug also were observed. On one instance the penicillin was injected one-half hour before delivery and the presence of the drug again was observed in the maternal and placental cord blood. The amniotic fluid was tested, but no penicillin was found to be present. A final case received the drug one hour before delivery and here again the presence of the drug was detected in the maternal blood but none was found in the placental cord blood.

Packaging

At the present time the drug is packaged in vials containing 5,000 to 1,000,000 units. For intramuscular and intrathecal injection 20 cc of physiological saline solution or 5 per cent glucose in saline are added to the contents of the desired number of units per dose. For intravenous administration the contents of a 100,000 unit vial are dissolved in 100 cc. of solution. In general, 90,000 units in a 24 hour period is the recommended dosage for streptococcus infections and it is administered intramuscularly in 15,000 unit doses every four hours. If the intravenous route is used the dosage is decreased to 7,500 units and injected every two hours. Staphylococcus infections have proven more resistant and require 200,000 units daily, given intramuscularly in 25,000 unit doses. This is then followed by 5,000 unit doses every one-half hour for a total of 240,000 units daily. Penicillin has given excellent results in experimental animals with clostridial infections, particularly those causing gas gangrene. With the administration of large doses of about 400,000 units per day penicillin has been used successfully for the treatment of gas gangrene in man. There have been no harmful effects when as much as 600,000 units of penicillin were administered within 24 hours.

Continued Treatment

It is recommended that after the patient is afebrile, treatment with one-half the regular dosage should be continued for at least one week. Due to the fact that some organisms become “penicillin fast” when small doses are given over a long period of time, it is advisable to give the drug in larger doses at the beginning. As much as 4 million units of penicillin have been required for treatment of patients with severe infections, but on the average 1 million units will treat the usual case. When treating patients with penicillin the clinical response should be assessed by the temperature, pulse, changes in symptoms, the results of blood culture and the effect on the local infection. However, it is strongly stressed that when facilities are available, the amount of penicillin in the blood should be titrated by one of the several known tests to determine whether sufficient amounts of the drug are being given.

In 1934 Hamre, et. al., reported on the toxicity of penicillin when administered by various routes to mice, guinea-pigs, and rabbits (8). This particular study announced that the acute toxicity of penicillin for these animals was low; about 100,000 Oxford units per kilogram, given intravenously, caused a severe reaction and death. They further state that 7,000 to 12,000 units of penicillin per day given subcutaneously over a period of several days, caused the death of guinea-pigs, but not of mice or rabbits. A dose of 1,000 units per kg. administered by the same route for 20 days did not kill the guinea-pigs. The animals that succumbed to the toxic doses of penicillin were carefully examined and the only lesions found were present, in three cases, in the wall of the left ventricle of the heart and foci of necrosis were present in the livers.

Toxicity

More recent work tends to point out that the toxicity of penicillin is not as low as previously stated. In October of 1944 Ercoli and Lafferty (4) carried on an investigation for the purpose of establishing the minimal healing and sterilizing doses for penicillin in experimental spirochetal infections. For the experiments, rabbits were infected intratessicularly 8 weeks before treatment with a strain of Treponema pallidum. These animals, before treatment was started, showed charcriform lesions containing large numbers of spirochetes and also orchitis of both testicles. Penicillin (Na salt), the potency of which varied from 100 to 300 units per

The Veterinary Student
mg., was used for treatment and injected intravenously. None of the rabbits apparently showed any toxicity. The orchitis had a definite regression 4 or 5 days after the first treatment. Another test rabbit was administered the same single dose five times at two hour intervals for a total dosage of 150,000 units/kg. and after a week's time the lesions as well as the orchitis were healed.

Recently work was published dealing with the experimental use of penicillin and proamidine in acute purulent arthritis. Rabbits were used for the experimental animals and sulpha compounds were first tested in the treatment of the ailments. However, these drugs did not possess the required potency for complete healing so penicillin was used. Propamidine was used to test against penicillin and although it was just as successful in producing sterile joints, it proved to be too toxic for all the animals. The treatment with penicillin proved successful in producing sterile joints and the only noticeable damage to the joint was a slight loss of luster.

Canine Meningitis

In 1943 Pilcher, Cobb, and Meacham published their study on the intravenous treatment of experimental Staphylococcus aureus meningitis in dogs and according to their results penicillin had little if any beneficial effect. The authors first injected from 50 to 500 units of penicillin in saline intracisternally in normal dogs. Transitory meningeal reactions (pleoeyctosis), the intensity of which was roughly proportional to the dose, were produced by single injections of penicillin in saline when administered intracisternally into normal dogs. The severity of the reactions was increased only slightly when doses were repeated at 24 hour intervals. Neurological or systemic disturbances were not observed and the animals remained normal in all their actions. In the infected animals 50 units of penicillin were administered once daily intrathecally. Larger and more frequent doses were also well tolerated. Of the control animals only one of 15 recovered but in the treated animals 13 out of 32 recovered. Support was added to the theory of the bacteriostatic action of penicillin by the fact that for several days the cerebrospinal fluids remained bacteriologically positive in the treated animals that recovered. Pilcher et al. considered that the intrathecal administration of penicillin will be of value in clinical meningitis.

Published Results

Published experimental results tend to indicate that penicillin possesses only mild cytotoxic properties. At a concentration of 1:1,000, leucocytes were immediately killed. In the same series of experiments on leucocytes a concentration of 1:500 of sulfanilamide showed only feeble pseudopod formation and no movement after two hours. Fibroblasts of the chick embryo heart were shown to be slightly toxic to concentrations of crude penicillin 1:6,000. Before irreversible toxic changes occurred it was necessary to expose the fibroblasts to a concentration greater than 1:6,000 for a period of 48 hours. It can be concluded from these data that significant toxicity to living cells does not result from concentrations of crude penicillin of less than 1:5,000.

Studies by Herrell and Heilman (9) compared the cytotoxic properties of various agents to penicillin. These studies proved that the antibactericidal properties of penicillin were superior to any of the others tested. Toxicity comparisons showed penicillin to be greatly superior in this aspect. A direct comparison with gramicidin on a weight for weight basis showed penicillin to be about one-tenth as toxic to migrating cells as the former substance.

Administration

It will be noted that in all the experiments and cases discussed the time interval between administering the penicillin doses was relatively short. If penicillin was being used by the average practitioner this would prove to be a difficult problem to overcome for it is not a drug that can be left with the farmer to be administered according to directions. This problem is one of the many that are facing the research workers in the veterinary
field of today. After the war, production of penicillin will be great enough to supply the demands of all, but it will be only through the diligent efforts of these workers that the treatment of animals with penicillin will become popular among the practicing veterinarians.

BIBLIOGRAPHY


Blood Transfusion

(Continued from page 147)

In order to produce a pint of milk it is necessary for 400 pounds of blood to pass through the udder. In producing 3 gallons of milk a day 5 tons of blood must pass through the udder.

156

The Veterinary Student