PENICILLIN BLOOD LEVEL STUDIES IN DAIRY CATTLE

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PENICILLIN BLOOD LEVEL STUDIES IN DAIRY CATTLE

Penicillin Blood Level Studies In Dairy Cattle

By

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INTRODUCTION

One of the major problems confronting the veterinary practitioner is the proper use of penicillin in cows. Different types of penicillin are now on the market for use by veterinarians, such as; amorphous, and procaine G alone and in various vehicles to delay absorption. There is a need for determining the penicillin blood level curves obtained when these various penicillin products are used in cattle. This report presents such data.

If penicillin is to be used effectively against susceptible organisms a basic therapeutic blood level must be attained and maintained.

REVIEW OF LITERATURE

Veterinary literature contains many references on the use of penicillin in diseases of animals. Some of the reports are favorable to its use while others report that penicillin has little or no value in treating the same disease. Obviously, there is much to learn concerning the proper use of penicillin or else the above descrepancies would not exist. During the past four years, since penicillin was released for veterinary use on April 21, 1945, a few scattered reports of penicillin blood levels in large and small animals have been reported by various workers.

The discovery of penicillin by Fleming (21), the success of its thereapeutic use in human streptococococcic infections since that time, its lack of irritating properties to the human, and the favorable report of Kakavas (33) on the use of crude preparations in streptococococcic mastitis stimulated the study of penicillin in various diseases of animals caused by penicillin susceptible organisms. Parkins, Wiley, Chendy and Zintel (45) report that the use of collodial gelatin as a vehicle with the addition of privine or neosynephrine had a definite action on sustaining blood levels in the dog and in man. In comparison with physiological saline as a vehicle, colloidal gelatin sustained the level twice as long, and colloidal gelatin plus privine or neosynephrine sustained the penicillin level about three times as long.

Loewe, Alture-Werber and Rosenblatt (38) studied the possibilities of rectal administration of penicillin. Varying amounts of sodium salt of penicillin were incorporated by hand in a simple

cocoa butterbase. Suppositories were inserted rectally without any previous preparation in hospitalized in-bed patients, selected at random, and in healthy ambulatory volunteers. Blood samples were withdrawn hourly for twelve hours and after twenty-four hours for penicillin assays. Appreciable penicillin blood levels were obtained in 12 out of 14 instances. Effective levels were found after twenty-four hours in 2 instances and for four to twelve hours in an additional 7 subjects.

The comparative therapeutic efficacy of different types of penicillin was studied by Spencer and Kraft (55). The combined result of all types of penicillin was an elimination of udder infection from 116, or 65 per cent, of 179 quarters when these quarters were treated but once. The two F penicillins cured 61 and 76 per cent of 54 and 17 quarters, respectively, or a combined average of about 65 per cent. The corresponding result for crystalline penicillin G was 69 per cent of 71 quarters. The penicillin K. eliminated infection from 12 of 22 quarters, or 55 per cent. Clinical observations on treated animals indicated the penicillin F. was approximately the same in irritating effects as the crystalline penicillin G. The crude penicillin produced a greater inflammatory reaction in the quarters than the purified types, but no persistent damage was observed.

In this study penicillin blood plasma levels of 1 to 4 units/cc. were obtained in cows when penicillin @ 1000 u/lb. body weight was administered intramuscularly. This confirmed the preliminary results of Foley and Lee (25) and Spencer and Kraft (55).

Foley and Lee (24, 25) showed that Streptococcus agalactiae was not killed by penicillin when exposure was made under conditions that did not favor growth (4° C.). Killing occurred at a slow rate when the streptococci were multiplying sluggishly (20° C.) and at a very rapid rate when they were multiplying rapidly (37° C.). All the surviving streptococci remaining in the 20° C. exposure tubes were killed promptly when incubated at 37° C. at which temperature growth could proceed at a rapid rate.

It would be expected, therefore, that best results in the treatment of streptococcus udder infection would be obtained when a therapeutic level is maintained in the udder for a period sufficiently long to ensure that none of the streptococci have failed to divide. In other words, since penicillin is fissibactericidal, a minimum effective concentration of the agent must be maintained in the milk until all the streptococci become susceptible during growth processes or multiplication and have been killed. These workers reported from in vitro studies that the therapeutic concentration of penicillin for S. agalactiae was 0.5 u/cc. The therapeutic concentration is defined as the amount of penicillin necessary to kill multiplying organisms. In contrast, Packer (43, 44) reported that the therapeutic concentration varied greatly according to the organism concerned, in some cases this was as low as 0.125 u/cc.

The desirability of therapeutic blood plasma levels for long periods of time was thereby established. Consequently Romansky and Rittman (52, 53) reported a method of prolonging the action of calcium

penicillin by suspending it in a mixture of beeswax and peanut oil, and administering it by the intramuscular route. The following year, Nichols and Haunz (41) showed that other vehicles delayed absorption from the place of injection and thus prolonged the blood level of the patient treated. These results with penicillin confirmed the previous reports of Code (13, 14) and his colleagues that beeswax and sesame oil prolonged the action of histamine and desoxycorticosterone acetate.

The suspension of penicillin in beeswax peanut oil mixtures was found to be a practical and effective method of delaying the absorption of penicillin after intramuscular or subcutaneous administration. At least 0.03 of an Oxford unit of penicillin per cc. can be maintained in the blood for at least twenty-four hours following a single intramuscular injection of 300,000 units of calcium penicillin in a 4.8 per cent mixture of beeswax and peanut oi. The view is generally held that when given by mouth penicillin is destroyed by stomach acid and in consequence only very small amounts can be detected in the urine (Rammelkamp and Keefer (49). Gyorgy (28, 1) observed that gonorrhea patients offered the best approach for the therapeutic evaluation of penicillin when given by mouth. Urinary excretion of penicillin is not an accurate yardstick of the therapeutic effect penicillin might exert while passing through the body. On the other hand, the fact that penicillin given by mouth appears in the urine proves that it is absorbed from the gastronintestinal tract.

Penicillin given by mouth in combination with a suitable buffer

salt, such as trisodium citrate, was found to be therapeutically effective in a number of cases of gonorrhea and other diseases. The rapid cure of gonorrhea by injected penicillin gave a reliable basis of comparison. In view of the discrepant statements concerning the mode of action of penicillin Lee, Foley and Epstein (24, 25, 37) considered it worth while to repeat and extend the work of Hobby et al. The experimental data indicated that multiplication of the organism is necessary for the killing action of penicillin. The work showed that multiplication of staphylococci takes place in the presence of 1.5 Florey units of penicillin per cc. and that the growing organism appears to be necessary since young staphylococci exposed to penicillin at a low temperature for a few hours were not killed, though when kept at the same low temperature for a week, in which time some division in the controls took place, penicillin was effective in killing the organisms. This report confirmed the work of Hobby, Meyer and Chaffee (31).

Boger (6) assayed each of 104 individuals to determine the average concentration of penicillin in plasma. The therapeutic plasma penicillin concentration desired for a human patient may be determined by a consideration of the penicillin sensitivity and the known or suspected localization of the infecting organism in the patient's body. Penicillin may be ineffective where the infection is in a joint not readily reached by the muscular system even though the organism may be readily susceptible to penicillin. If the organism is quite sensitive to penicillin but the focus of infection is well protected from direct contact with the blood stream,

the therapeutic penicillin plasma concentration will of necessity have to be greater than would be required if the infecting organism was more directly exposed to the antibiotic agent present in the body fluids.

Boger (6) showed that the effect of caronamide when administered by mouth is to elevate the penicillin plasma concentrations from two to seven fold. It does this by inhibiting the penicillin transport system of the renal tubules. Caronamide plasma concentration of 15 mg. per 100 cc. probably is a critical level below which a two-fold enhancement effect on penicillin plasma levels cannot be regularly expected in patients with normally functioning kidneys. Three grams every three hours will maintain plasma caronamide concentrations between 20-40 mg. per 100 cc. in the majority of patients, and these concentrations will produce maximal inhibition of tubular excretion of penicillin. Occasionally, nausea and vomiting have been observed, most of which has been due to mechanical irritation due to swallowing the larger dose of drug.

Fisk, Foord and Allis (22) compared the serum levels obtained in rabbits after injections of penicillin in physiological saline containing adrenaline. Using saline as a vehicle, measurable levels were maintained for approximately 1.5 hours, whereas, saline plus adrenaline maintained a measurable level for 3 to 4 hours. The addition of adrenaline was observed to level off the peak and prolong the duration of the blood concentration curve. Similar results were obtained in human patients. In these experiments saline used as a vehicle maintained a measurable level for approximately 2 hours while saline plus adrenaline maintained it for approximately 2.5 to

3 hours.

Hobby, Meyer and Chaffee (30, 31) experimented to determine the mode of action of penicillin, the results of which indicate that the penicillin may act either as a bacteriostatic or as a true bactericidal agent. Thus the action of this agent is similar to that of gramicidin and tyrocidin, which is in contrast to that of the sulfonamides which cause a slowing up of the rate of growth. The action of the penicillin on hemolytic streptococci is not accompanied by lysis of the organisms. Penicillin is apparently able to destroy the organism only if active multiplication takes place.

Hobby, Meyer and Chaffee (21, 30) tested the activity of penicillin in vitro by the dilution method against a wide variety of organisms. Confirming the work of Florey and his co-workers (23), the preparations tested were found to exert a remarkable effect in vitro on many of the Gram positive organisms, both aerobic and anaerobic, as well as on bonococci and menigococci. All Gram negative organisms tested were relatively insusceptible to the action of this drug.

The work of Rammelkamp and Keefer (49) on the antibacterial effect of blood noted after penicillin administration, indicates that this substance should prove to be an exceptionally effective agent in the treatment of staphylococcal and hemolytic streptococcal infections in man.

In general, blood or serum containing adequate amounts of penicillin killed all the organisms, in the largest inoculum of hemolytic streptococci used. This is in distinct contrast to the action

of adequate amounts of penicillin in blood or serum, on Staphylococcus aureus, since with this organism all the bacteria are not killed upon the addition of a large inoculum. This observation may have an important bearing on the use of penicillin in the treatment of staphylococcal infections in man and suggests that adequate concentrations of the antibiotic substance should be maintained in the blood stream for a long period of time in order to ensure the complete killing of all staphylococci.

Hobby has shown that penicillin exhibits a greater antibacterial effect than sulfathiazole when tested against the hemolytic streptococcus in broth culture. The present studies have demonstrated that when penicillin is added to whole defibrinated blood, the antistaphylococcal and antistreptococcal effect was greater than when the sulfonamide drugs were added. Similar results were obtained on testing the blood of normal subjects after the administration of penicillin or sulfadiazine. It is important to point out, however, that the antibacterial action of blood after a single injection of penicillin in man is of relatively short duration, since it is excreted rapidly in the urine and a small amount is destroyed in the body.

The degree of antibacterial action observed in whole blood after the administration of penicillin was directly related to its concentration in the serum. Therefore, in the treatment of clinical infections, penicillin must be given frequently and in adequate doses in order to maintain sufficient concentrations in the body to exert an antibacterial effect.

Doll and Dimock (17) found that single intramuscular injections of 300 to 500 units of penicillin per pound of body weight given in a suspension of beeswax and peanut oil to horses maintained effective blood concentration for four to six hours. This interval was approximately double that when saline solutions were used as a vehicle.

Rammelkamp and Keefer (48) determined the absorption, excretion, and distribution of penicillin when administered by various routes. Intravenous injections of penicillin resulted in high initial concentration in the blood plasma which was followed by an abrupt fall. Traces of penicillin were found in the blood for 30 to 210 minutes after the injection, the length of time depending on the amount administered. The sharp fall noted in the serum concentration immediately after injection was associated with an increased excretion in the urine. The average excretion after intravenous injection was 58 per cent of the administered dose.

A penicillin level was rapidly obtained when given intravenously and slowly obtained when given by intramuscular or subcutaneous injection. Excretion in the urine was rapid following intramuscular injections and delayed after subcutaneous injections.

Absorption from the body cavities was delayed, and this was reflected in the slow excretion of penicillin by the kidneys. The total amount in the urine was somewhat lower than that obtained following intravenous injection. Fluid aspirated from the pleural and joint cavities, 13 and 22 hours after the injection, showed appreciable amounts of penicillin remaining.

In the presence of renal failure, penicillin was not excreted rapidly, and as a result, high concentrations were maintained in the blood stream after intravenous injections.

Studies on the distribution of penicillin showed that the substance failed to pentrate the red cells in significant amounts. In general, the average concentration found in erythrocytes was less than 10 per cent of plasma concentration. No penicillin was found in the spinal fluid, saliva, or tears, in subjects receiving it intravenously.

Bigger (4) investigated the in vitro antibacterial effect of penicillin combined with various sulfonamides. The presence of sulfathiazole in broth greatly enhanced the inhibitory action of penicillin on staphylococci. It is suggested that this synergistic action of sulfonamides and penicillin should be employed in the treatment of suitable infections in man.

The efficacy of various agents for delaying absorption of penicillin in the horse was reported by Doll and Dimock (17). They point out that the absorption of penicillin was somewhat erratic when vasoconstrictors were used in conjunction with physiological saline, saline and dextrose or gelatin solutions. Since the vasoconstrictors produced only a short prolongation of the effective blood level period, the authors consider them as offering very little advantage in the therapeutic use of penicillin. Their results also indicate that the use of dextrose or gelatin in combination with vasoconstrictor drugs, offers no significant advantage over saline preparations.

Doll and Hull (18) found that an emulsion of water-in-oil as a vehicle for the intramuscular administration of penicillin produced no delay in absorption as compared with physiological saline as a vehicle in horses and sheep. On the basis of 500 units per pound of body weight the saline vehicle maintained a measurable level for 2.5 to 3 hours, and in the case of water-in-oil emulsion the level was maintained for 3 hours in horses. In sheep, on the basis of 500 units per pound of body weight, saline maintained the level for 4 hours and water-in-oil emulsion maintained the level 4 to 5 hours.

Watts and McLeod (57) determined penicillin levels in blood serum and milk of bovines after intramuscular injection. The sensitivity to penicillin of the pathogenic cocci associated with diseases of the bovine mammary gland indicates the possible usefulness of the agent in the treatment of such diseases.

The inhibitory effect of bovine serum on the action of bacteriostatic drugs is not peculiar to penicillin for sulfanilamide is similarly influenced. There is, however, a difference between the two drugs, for heating the serum at 56° C. for 30 minutes destroys almost completely the anti-penicillin activity whereas such treatment does not effect the anti-sulfanilamide activity. Thus it appears that some heat labile factor or factors present in bovine serum act specifically against penicillin. That such a factor is very labile is clearly evidenced by its inactivation by mild heat-treatment and also by the fact that serum kept overnight tends to lose its anti-penicillin activity. If this anti-penicillin action of fresh serum occurs in vivo as well as in vitro, the fairly high

concentration of the drug found in the bovine serum in the present work would have little therapeutic value.

Brinker (8) reported on the penicillin blood plasma levels in dogs following the administration of various doses (250, 500 and 1000 units per lb.) in 5 per cent dextrose and physiological saline given intramuscularly and subcutaneously. In this series of trials the resulting levels following subcutaneous administration were found to be slightly superior to those following intramuscular administration both in concentrations and prolongation of the plasma levels. A concentration of 0.03 units or better was found to be maintained an average of 4 hours following a single injection of 1000 units per pound, 3.5 hours following 500 units per pound and 2.25 hours following 250 units per pound.

Five per cent dextrose in physiological saline was found to be equal or slightly superior to the water-in-oil vehicles in maintaining penicillin plasma levels. Romansky's formula was found to excell the above vehicles, as a level of 0.03 units or better were maintained in all cases at least 6.5 hours following a subcutaneous injection of 1000 units per pound.

Bryan, Horwood, and Huffman (9) on intravenous administration of 50,000 units of penicillin at three-hour intervals until 200,000 units were given was in-effective in the treatment of chronic S. agalactiae mastitis infection of cows. Individual quarters infused with 1,000, 5,000, 10,000 and 20,000 units, respectively, recovered with one treatment.

Klein, Crisman, and Moor (36) experimented with penicillin on staphyloccocic infection of the cow's udder. It was found that the

antibacterial action of the penicillin continued for twelve hours and that four injections at twelve-hour intervals were as effective as eight at six-hour intervals, although a more prolonged antibacterial action appeared to be obtained by eight rather than four injections. The organisms disappeared from the milk during treatment, but only one of the quarters was permanently freed from infection, while in another quarter the original strain of staphylococcus was replaced by one less pathogenic.

Creip (16) reported allergy to penicillin in a human patient ten days after receiving a course of penicillin totaling 2,800,000 units. This patient was started on a second course during which he received 120,000 units a day for seven days. Massive generalized uritcaria developed after the first dose of the second course of penicillin and persisted until the drug was ciscontinued. Positive skin tests, positive passive transfer, and positive precipitin tests indicated the presence of some immune substances, and such as reagins and precipitins, in the patient's serum, but their role in mediating the reaction is not clear. This confirms the work of Lyons (39), Keefer (34) et al, on the toxicity of penicillin.

Heilman and Herrell (29) studied the effect of penicillin in vitro and in vivo or Erysipelothrix rhusiopathiae, the organism which causes swine erysipelas and also erysipeloid in man. On in vitro studies the organism proved to be sensitive to penicillin. Controlled experiments then were made in mice inoculated with a virulent culture of E. rhusiopathiae. In a group of 40 untreated animals, the mortality rate was 100 per cent, as compared with 5 per

cent, as compared with 5 per cent in the 40 that received penicillin. From these results, Heilman and Herrell believe penicillin should prove effective in the treatment of erysipelas in swine and of erysipeloid in man.

Abraham, Chain, Fletcher, Gardner, Heatley, Jennings and Florey (1) observed the growth in vitro of many pathogenic bacteria is prevented by purified penicillin at a dilution of one in a million, or more, Other, mainly Gram negative organisms, possess lessor degrees of sensitivity, some being quite resistant. Cultivation of a strain of Staph. aureus for about four months in the presence of penicillin enabled it to grow in a concentration of penicillin a thousand times greater than that which almost completely inhibited the parent strain, but no penicillin-destroying enzyme was found. The bacteriostatic power of penicillin against streptococci and staphylococci is much greater than that of the sulfonamides and is only slightly influenced by the number of bacteria to be inhibited. Blood, pus, and tissue derivatives do not prevent its action. This is of great importance in heavily infected wounds in which sulfonamide drugs have little beneficial action.

Experiments show that penicillin is less toxic than the sulfonamides to leukocytes in vitro; concentrations much higher than those required for bacteriostasis do not impair the activity of the leukocytes. In tissue culture penicillin concentration of 1:5,000 is not significantly toxic to living cells, and there is no secondary reaction.

Beyer, Woodward, Peters, Verwey and Mattis (3) in experiments with dogs described the recovery of a dose of penicillin in the urine as greatly reduced when P-aminohippuric acid was administered. Forty-eight hour experiments, in which both penicillin and P-aminohippuric acid were infused continuously, showed that if the plasma level of the latter was maintained at 20 to 30 mg. per 100 cc. a plasma level of 0.08 to 0.1 unit of penicillin per cubic centimeter could be maintained by the infusion of 15 units a minute. Without P-aminohippuric acid the penicillin level was 0.02 units or less. Raising or lowering the plasma concentration of P-aminohippuric acid resulted in raising or lowering the penicillin content in the plasma even though the penicillin was infused at a constant rate. No pathologic findings attributable to P-aminohippuric acid were observed.

Doll and Wallace (19) in a comparative study on horses and sheep used a water-in-oil emulsion, saline and beeswax to delay absorption of penicillin. The penicillin was the potassium salt of crystalline G. It was dissolved in saline before emulsifying with the oil vehicle. One product labeled penol diluent, containing oxycholesterol in vegetable oil, was combined in the proportion of 3.0 cc. of penol with 100,000 units of penicillin dissolved in 1.0 cc. of saline. Another product labeled pendil-improved formula, containing cholesterol derivative 11 parts, and peanut oil 20 parts W/V, with beeswax 2 per cent W/V, was combined in various ways with penicillin dissolved in saline.

With the penol diluent, the results were slightly superior

to those observed previously with a water-in-oil plus wax vehicle, in which the period of detectable blood level did not exceed three hours for horses nor five hours for sheep. The longer duration of the blood level in sheep appears to be of no significance in comparison with the penol diluent, as sheep have been found to maintain measurable blood levels for one to two hours longer than horses following doses of 500 units per pound in saline solution administered intramuscularly.

The most significant evidence of delayed absorption from the pendil menstrum was the prolonged period of detectable blood level, which varied in most trials between eight and ten hours.

With the same dosage of penicillin in units per pound of body weight, the pendil maintained measurable blood levels from one to seven hours longer than the penol diluent; from two to eight hours longer than the water-in-oil plus wax vehicle, previously investigated; and from three to ten hours longer than penicillin in aqueous solution. With the combination, pendil, 3.0 cc., and penicillin, 100,000 units in 1.0 cc. of saline, the serum concentrations were comparable in duration in hours and in units per cc. to those obtained with penicillin in oil and wax, (Romansky formula, 300,000 units per cc.).. However, the serum concentrations with pendil tended to run at higher levels during the first two hours than with the Romansky formula.

The improvement in penicillin products available prompted Bolton et al. to determine the amount of procaine penicillin remaining in the treated quarter at varying time intervals after treatment. Procaine penicillin in bougie form was administered as

follows; 18 cows received 25,000 units per quarter, 6 received 50,000 and 6 received 75,000 units per quarter. The number of Oxford units in the milk at the end of eight hours was far in excess of that required to inhibit the growth of streptococci. The use of 25,000 units in each infected or suspected quarter gave results as satisfactory as when twice or three times that amount was used.

The normal ranges of the bovine blood constituents reported by Coffin (15) were as follows: erythrocytes 5.0 to 10.3 millions per cu. mm., leukocytes 5 to 12 thousands per cu. mm., hemoglobin 8.0 to 14.5 gms. per 100 cc., neutrophiles .12 to 4.8 thousands per cu. mm., basophiles 0 to .1 thousands per cu. mm., lymphocytes 2.7 to 6.9 thousands per cu. mm., monocytes .15 to 1.8 thousands per cu. mm.

The normal ranges of bovine blood constituents reported by Duke (20) were as follows: erythrocytes 6,325,000 per cu. mm., leukocytes 7,900 per cu. mm., hemoglobin 12.03 gms. per 100 cc., neutrophiles 21 per cent, monocytes 10 per cent, lymphocytes 64 per cent, eosinophiles 5 per cent and basophiles 0 - 1 per cent.

METHODS

The proccedures used in these experiments were as follows:

Estimation of Penicillin in Body Fluids

Although several methods have been reported (2, 26, 35, 47) for measuring the penicillin blood plasma levels the official test of the Food and Drug Administration, developed by Randall, Price and Welch (51) in 1945 was used. The technique was as follows: one-half cc. amounts of sterile yeast beef broth* were placed in regular size sterile test tubes. Serial dilutions were made by adding one-half cc. of the blood plasma to the first and second tubes of the series and then transferring one- half cc. in serial dilution for as many tubes as necessary (usually 10 to 15 tubes). The first tube in the series contained one-half cc. of the material under test only. A standard was prepared for comparison by diluting a known potency penicillin** to one unit per cc. in sterile water. This one-unit standard was diluted exactly as above in serial dilution. One and one-half cc. of a 1:100 dilution of the test organism*** in yeast beef broth was added to all tubes; then the tubes were incubated at 37° C. for 18 hours. The last tube in which no growth occurs is taken as the end-point. This was usually sharp, inasmuch as one tube was clear while the next one in the

*Difco Yeast Beef Broth Dehydrated, Difco Lab., Detroit.
**Reference Standard of known potency was obtained from Food and Drug Adm.
***Original culture of test organism (B. subtilis) was obtained from Food and Drug Administration.

series would have the typical pellicle of B. subtilis on the surface of the medium.

The concentration of penicillin in the unknown was then determined by comparing the end-point of the unknown with that of the standard. Thus if an unknown has an end-point of tube 6, it contains 1 unit of penicillin per cc., if end-point is 5 or 7, it contains 0.5 and 2 units per cc., respectively, because generally tube 6 is the endpoint for the standard. Ordinarily the test as described here in sufficiently sensitive to determine potencies as low as 0.03 units of penicillin per cc. of blood plasma.

Determination of Hemoglobin. The Sheard - Sanford Photelometer was used in determining the grams of hemoglobin per 100 cc. of blood (54). Preparation of the sample of blood was as follows: twenty cc. of the diluting fluid (0.1 per cent solution of sodium carbonate in water) was measured accurately into a suitable container. To this was added 0.1 cc. of blood. The mixture was thoroughly shaken and then transferred to the photelometer for reading using a green filter. This reading was then converted into grams of hemoglobin per 100 cc. of blood.

Leukocyte and Erythrocyte Enumeration. Leukocyte and erythrocyte counts were made at the time of each bleeding. The counts were made within 24 hours after bleeding using Thoma diluting pipettes and Bright-line Improved Neubauer Counting Chambers (15). One tenth normal hydrochloric acid was used as the diluting fluid for counting leukocytes, while Leak and Guy diluting fluid, was used for making erythrocyte counts.

Differential Leukocyte Counts. Blood smears were prepared within one hour after bleeding by the two-slide method and stained with Wright's stain (15) immediately following drying.

The anticoagulant used in collecting blood samples was the dried residue of 0.2 cc. of 20% sodium citrate in each tube for 5 cc. of blood.

All needles were sterilized after each bleeding using heat sterilization to avoid chemical disinfectant which might interfere with penicillin plasma levels. In all of the series of experiments blood studies including white blood count, red blood count, hemoglobin and leukocytes differential counts were made. In Tables 12 to 23 it may be noted that no significant variation appeared in the blood picture during the administration of penicillin. Thus one can conclude that none of the penicillins or vehicles used were toxic to the cow.

RESULTS AND DISCUSSION

The problem of immediate importance was to determine if the amount of anticoagulant used (0.2 cc. of a 20% sodium citrate for 5 cc. of blood) had any effect on inhibiting the growth of B. subtilis, the test organism. This was determined by setting up trials runs using penicillin blood plasma against the standard. Seven series of ten tubes each were set up. To each of tube 1 and 2 was added 0.5 cc. of blood plasma. A standard (1 unit cc.) was prepared for comparison by diluting a known potency penicillin. (51).

The results were the same as the standard (F. D.A.), therefore the sodium citrate in no way influenced the results obtained. Brinker (8), Chandler, Price and Randall (12) reported that humans and dogs had an inhibitory factor against B. subtilis, however, the inhibitory substance was both variable and transitory in a given individual.

In the actual testing for the blood plasma concentration of penicillin a sufficient number of hourly blood samples were drawn so that in every case the series of samples on an individual acted as a check on the presence or absence of inhibitory factor even though a sample was not drawn in each case, specifically for this purpose prior to injecting the penicillin no inhibitory action was found

The blood plasma levels obtained by the subcutaneous, intramuscular, intravenous and intraperitoneal administration of 300 units per pound of body weight of crystallin penicillin G, are

presented in Graph 1, and Tables 1, 2, 3, 5. Following intravenous injection a level of 0.25 units cc. of plasma was obtained at the one hour sampling and a measurable level persisted for only 2 hours. This confirms in cows, the results obtained by Brinker (8) in dogs.

The intraperitoneal administration of penicillin G, sodium salt, in sterile water resulted in a blood plasma level of 1.0 unit cc. at the one hour sampling. The plasma level gradually declined to 0.06 units within four hours. This level is approximately the same as that obtained with the use of Romansky's Formula at the same level of administration (subcutaneous). Graph 3 and Table 10.

The intramuscular administration gave a rapid blood plasma level of 1.0 unit per cc. in one hour and continued to hold a level of 0.5 units for three hours and steadily declined to 0.06 units by six hours. A comparison of these blood plasma levels reveals that the subcutaneous administration is somewhat superior to the intravenous and intraperitoneal routes both as to the prolongation of blood plasma levels and the ease of administration. However, the plasma level of 1.0 unit is obtained by the first hour, but slowly declines to 0.06 at the end of five hours. The subcutaneous use of Romansky's Formula at the rate of 300 units per pound body weight yielded a higher blood plasma level than did crystalline penicillin G and a measurable level was maintained for one hour longer. This is in agreement with the result of Brinker.

The data of Graph 1 show that the intramuscular administration of crystalline penicillin G. gives the highest blood plasma levels and maintains the concentration up to 6 hours.

The blood plasma levels obtained in the cow when 500 units per pound of body weight of crystalline penicillin G was administered subcutaneously, intramuscularly, intravenously and intraperitonealy are presented in Graph 2. This Graph is a composite of the data of Tables 4, 6, 7 8. Intravenous injection (jugular vein) yielded an immediate concentration of 0.5 units per cc. of blood plasma within 1 hour. This rapidly declined to 0.06 in 3 hours. The intraperitoneal injection gave a blood plasma concentration of 1.5 units/cc. in 1 hour but failed to hold the level for more than 4 hours. When given by subcutaneous and intramuscular injection the blood plasma concentrations were similiar in the initial rise (up to 1 unit/cc.). After this the penicillin level decreased but a level of 0.06 was maintained for 7 hours. Thus the subcutaneous or intramuscular routes of administration are preferred because the blood levels of penicillin were maintained twice longer than by intravenous and intraperitoneal administration.

A comparison of blood plasma levels obtained in the cow when 300 units per pound of body weight of procaine penicillin G was given in aqueous solution and in oil and wax, and when 1500 units per pound of the same penicillin in aqueous solution are presented in Graph 4 and Table 9. All injections were made intramuscularly. The aqueous and oil and wax injection of 300 units per pound body weight compare very closely with each other in regard to the blood

plasma penicillin concentration reached at 1 hour, and to the gradual drop in concentration from this point until the penicillin was no longer measurable, which in each case was between 18 and 20 hours.

The highest concentration was obtained when 1500 units per pound of body weight of the aqueous solution of procaine penicillin G was injected. In this case the blood concentration rose to 1.0 unit per cc. in 1 hour and continued to hold this level for 3 hours, after which it dropped to 0.5 units for 4 more hours following which it gradually declined to 0.06 in 12 hours which level was maintained for the remaining 8 hours. Thus a level of 0.06 or higher was maintained for the total of 21 hours by a single injection.

With the improvement in preparation and purification of penicillin several procaine products have been put on the market. A series of experiments were run to determine the penicillin blood plasma levels following a single injection of 250 and 1000 units per pound body weight of procaine penicillin (S-R, aqueous). This data is shown in Graph 5 and Table 2.

In the series of trials 250 units per pound of procaine penicillin S-R was injected intramuscularly into a 160 pound calf. The blood plasma concentration was 1.0 units within 2 hours and remained at this high level for 30 hours, at which time no further samples were drawn. This is the highest blood plasma concentration obtained with any type of penicillin administered at this dosage.

The intramuscular injection of 250 units per pound into a 1000 pound cow gave a blood plasma concentration of only 0.5 units/cc. in 2 hours as compared to 1 unit in the calf. Although this level was maintained in the calf it gradually dropped to 0.06 during a 30 hour period in the cow.

In intramuscular administration of 1000 units per pound body weight to a 160 pound calf the blood plasma concentration was 2.0 units within 2 hours and steadily rose to 4.0 units by 10 hours when it gradually decreased to 0.06 units by 50 hours. Intramuscular injection of 1000 units per pound body weight of penicillin S-R into a 1000 pound cow compared very closely with the level obtained in the calf. The highest concentration, 4 units, was obtained at the 6 hour bleeding period, after which it gradually declined to 0.06 by the 50th hour.

Table 1. The blood plasma level in the cow when 300 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered subcutaneously.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.0	1.0	1.0
2	0.5	0.5	0.5
3	0.25	0.25	0.25
4	0.12	0.12	0.12
5	0.06	0.06	0.06
6	0.06	0.06	0.06
7	< 0.06	< 0.06	< 0.06

Table 2. The blood plasma level in the cow when 300 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered intramuscularly.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.0	1.0	1.0
2	0.5	0.5	0.5
3	0.5	0.5	0.5
4	0.25	0.25	0.25
5	0.12	0.25	0.12
6	0.12	0.25	0.06
7	0.6	0.12	0.06
8	0.06	0.06	0.06
9	< 0.06	< 0.06	< 0.06

Table 3. The blood plasma level in the cow when 300 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered intravenously.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	0.25	0.25	0.25
2	0.06	0.06	0.06
3	< 0.06	< 0.06	< 0.06

Table 4. The blood plasma level in the cow when 500 units per lb. body weight of penicillin G sodium salt, using water as the vehicle, was administered intravenously.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	0.5	0.5	0.25
2	0.25	0.25	0.06
3	0.06	0.06	< 0.06
4	< 0.06	< 0.06	

Table 5. The blood plasma level in the cow when 300 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered intraperitonealy.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.0	1.0	0.5
2	0.5	0.5	0.12
3	0.25	0.25	0.06
4	0.12	0.12	< 0.06
5	< 0.06	< 0.06	

Table 6. The blood plasma level in the cow when 500 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered intraperitonealy.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.5	1.0	1.0
2	1.0	0.5	0.5
3	0.5	0.25	0.12
4	0.06	0.06	< 0.06
5	< 0.06	< 0.06	

Table 7. The blood plasma level in the cow when 500 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered subcutaneously.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.0	1.0	1.0
2	0.5	0.5	0.5
3	0.25	0.25	0.25
4	0.12	0.12	0.12
5	0.12	0.12	0.12
6	0.06	0.12	0.06
7	0.06	0.06	0.06
8	< 0.06	< 0.06	< 0.06

Table 8. The blood plasma level in the cow when 500 units per lb. body weight of penicillin G sodium salt, using sterile water as the vehicle, was administered intramuscularly.

Hours After Injection	Cow Number		
	1	2	3
	Units of penicillin per cc of blood plasma		
1	1.0	1.0	1.0
2	0.5	0.5	0.5
3	0.5	0.5	0.5
4	0.25	0.25	0.25
5	0.25	0.25	0.12
6	0.12	0.25	0.06
7	0.06	0.12	0.06
8	0.06	0.06	< 0.06
9	< 0.06	< 0.06	

Table 9. The blood plasma level in the cow when 300 and 1500 units per lb. body weight of procaine penicillin G, oil and wax solution and aqueous solution were administered intramuscularly in the cow.

Hours After Injection	Units of penicillin per cc of blood plasma		
	Penicillin 1*	Penicillin 2*	Penicillin 3*
1	0.5	0.5	1
2	0.5	0.5	1
3	0.5	0.25	1
4	0.25	0.25	0.5
5	0.25	0.25	0.5
6	0.25	0.12	0.5
7	0.12	0.12	0.5
8	0.06	0.12	0.25
9	0.06	0.06	0.25
10	0.06	0.06	0.12
11	0.06	0.06	0.12
12	0.06	0.06	0.06
13	0.06	0.06	0.06
14	0.06	0.06	0.06
15	0.06	0.06	0.06
16	0.06	0.06	0.06
17	0.06	0.06	0.06
18	0.06	0.06	0.06
19	0.06	< 0.06	0.06
20	0.06	< 0.06	0.06
21	< 0.06	< 0.06	0.06
22	< 0.06	< 0.06	< 0.06

* Penicillin 1. Wycillin crystalline procaine penicillin G, oil and wax, 300 units per lb. body weight.
* Penicillin 2. Wycillin crystalline procaine penicillin G, aqueous, 300 units per lb. body weight.
* Penicillin 3. Wycillin crystalline procaine penicillin G, aqueous, 1500 units per lb. body weight.

Table 10. The blood plasma level in the cow when 300 units per lb. body weight of Romansky's Formula was administered subcutaneously.

Hours After Injection	Cow Number	
	2	3
	Units of penicillin per cc of blood plasma	
1	1.0	1.0
2	1.0	1.0
3	0.50	0.50
4	0.12	0.25
5	< 0.06	< 0.06

Table 11. The blood plasma level in the cow and calf when 250 and 1,000 units per lb. body weight of S-R penicillin was administered intramuscularly.

Hours After Injection	Cow		Calf	
	250 U/lb. body wt.	1,000 U/lb. body wt.	250 U/lb. body wt.	1,000 U/lb. body wt.
	Units of penicillin per cc blood plasma			
2	0.5	1	1	2
6	0.5	4	1	4
10	0.25	2	1	4
14	0.25	2	1	2
26	0.12	1	1	2
30	0.06	0.5	1	1
34	Not run	0.12	Not run	0.25
50	Not run	< 0.06	Not run	0.06

Table 12. Blood studies on cow 1 following the subcutaneous administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs. | 2/5/48

History - Apparently healthy | Vehicle - Sterile water | 5 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	7,800	6,190,000	11.3	8	-	22	58	12
2	0.5								
3	0.25	7,900	6,000,000	11.9	6	-	20	64	10
4	0.12								
5	0.6	7,600	6,780,000	11.7	4	-	22	61	13
6	0.06								
7	< 0.06	7,500	6,500,000	11.2	11	1	25	54	9

Penicillin U/cc. Plasma - Units of penicillin per cc. of blood plasma
W.B.C. - White Blood Cells per cu. mm.
R.B.C. - Red Blood Cells per cu. mm.
Hb. g./100 cc. - Grams of hemoglobin per 100 cc. of blood
E. - Eosinophiles B. - Basophiles N. - Neutrophiles L. - Lymphocytes M. - Monocytes

Table 13. Blood studies on cow 2 following the subcutaneous administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs | 2/8/48

History - Apparently healthy | Vehicle - Sterile water | 6 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	8,200	6,320,000	12.6	7	1	19	64	9
2	0.5								
3	0.25								
4	0.12	7,600	7,100,000	11.7	10	-	24	55	11
5	0.06								
6	0.06	7,900	6,580,000	12.4	6	-	20	64	10
7	< 0.06								

Table 14. Blood studies on cow 3 following the subcutaneous administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs. | 2/14/48

History - Apparently healthy | Vehicle - Sterile water | 7 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	9,000	6,600,000	12.5	5	-	21	65	9
2	0.5								
3	0.25								
4	0.12								
5	0.06	8,200	6,580,000	12.8	7	1	24	58	10
6	0.06								
7	< 0.06	8,600	6,550,000	12.6	9	-	20	63	8

Table 15. Blood studies on cow 1 following the intramuscular administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs. | 3/19/48

History - Apparently healthy | Vehicle - Sterile water | 5 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0								
2	0.5	8,300	6,890,000	11.3	5	-	23	61	11
3	0.5								
4	0.25								
5	0.12	7,800	6,000,000	11.9	9	-	21	62	8
6	0.12								
7	0.06	8,600	7,300,000	11.6	6	-	25	56	13
8	< 0.06								

Table 17. Blood studies on cow 3 following intramuscular administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs. | 3/24/48

History - Apparently healthy | Vehicle - Sterile water | 7 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	8,780	6,700,000	12.3	11	-	23	54	12
2	0.5								
3	0.5								
4	0.25								
5	0.12	8,340	6,800,000	11.9	9	-	22	59	10
6	0.06								
7	< 0.06								

Table 20. Blood studies on cow 1 following the intraperitoneal administration of crystalline penicillin G, sodium salt.

Dose - 300 U/lb. body weight | 1,000 lbs. | 3/26/48

History - Apparently healthy | Vehicle - Sterile water | 5 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0								
2	0.5	7,400	6,500,000	11.0	5	-	24	60	11
3	0.25								
4	0.12								
5	< 0.06	7,300	6,700,000	11.3	8	1	21	57	13

Table 22. Blood studies on cow 1 following the intramuscular administration of crystalline penicillin G, sodium salt.

Dose - 500 U/lb. body weight | 1,000 lbs. | 4/16/48

History - Apparently healthy | Vehicle - Sterile water | 5 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	7,300	6,580,000	11.5	6	1	21	60	12
2	0.5								
3	0.25								
4	0.25	7,400	6,520,000	11.9	4	-	23	60	13
5	0.12								
6	0.06								
7	0.06								
8	< 0.06	7,200	6,600,000	11.3	7	1	22	62	8

Table 23. Blood studies on cow 2 following the intramuscular administration of crystalline penicillin G, sodium salt.

Dose - 500 U/lb. body weight | 1,000 lbs. | 4/13/48

History - Apparently healthy | Vehicle - Sterile water | 6 yrs.

Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	7,500	7,510,000	12.4	9	2	19	57	13
2	0.5								
3	0.25								
4	0.12								
5	0.12	7,200	7,480,000	12.2	6	-	20	65	9
6	0.06								
7	0.06								
8	< 0.06	7,300	7,460,000	12.0	10	-	24	58	8

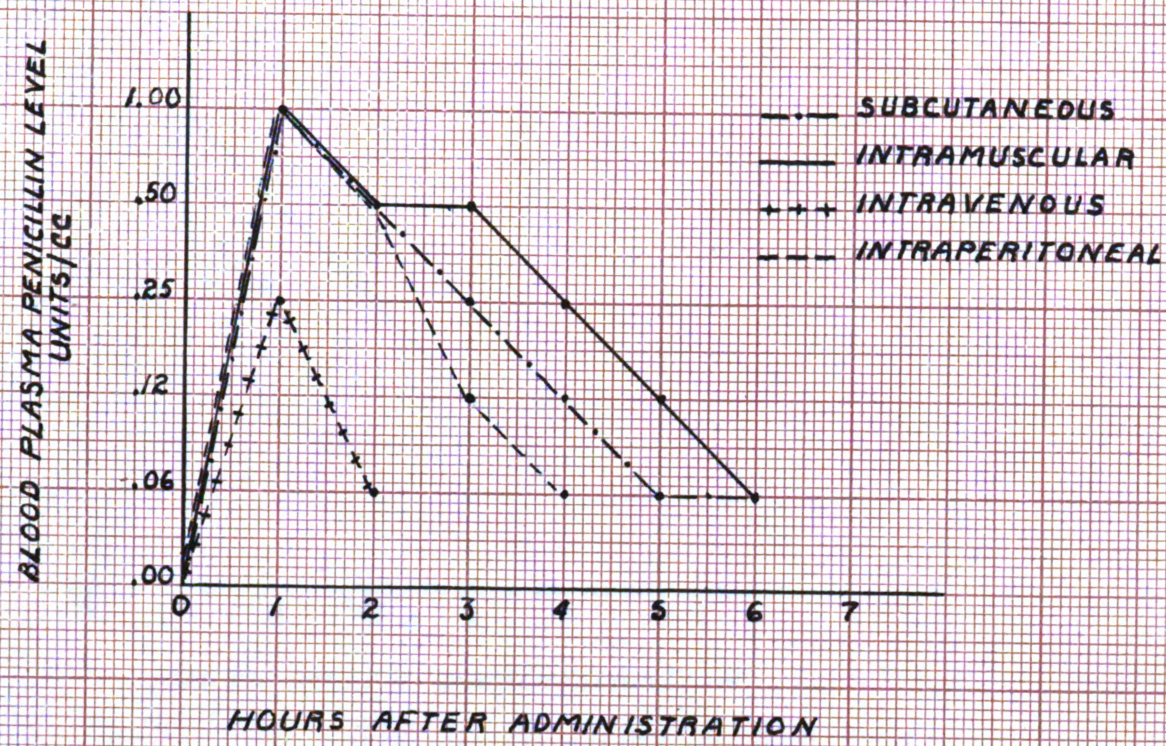
Table 24. Blood studies on cow 3 following subcutaneous administration of Romansky's Formula.

Dose - 300 U/lb. body weight 1,000 lbs. 4/9/48

History - Apparently healthy Vehicle - Romansky's Formula 7 yrs.

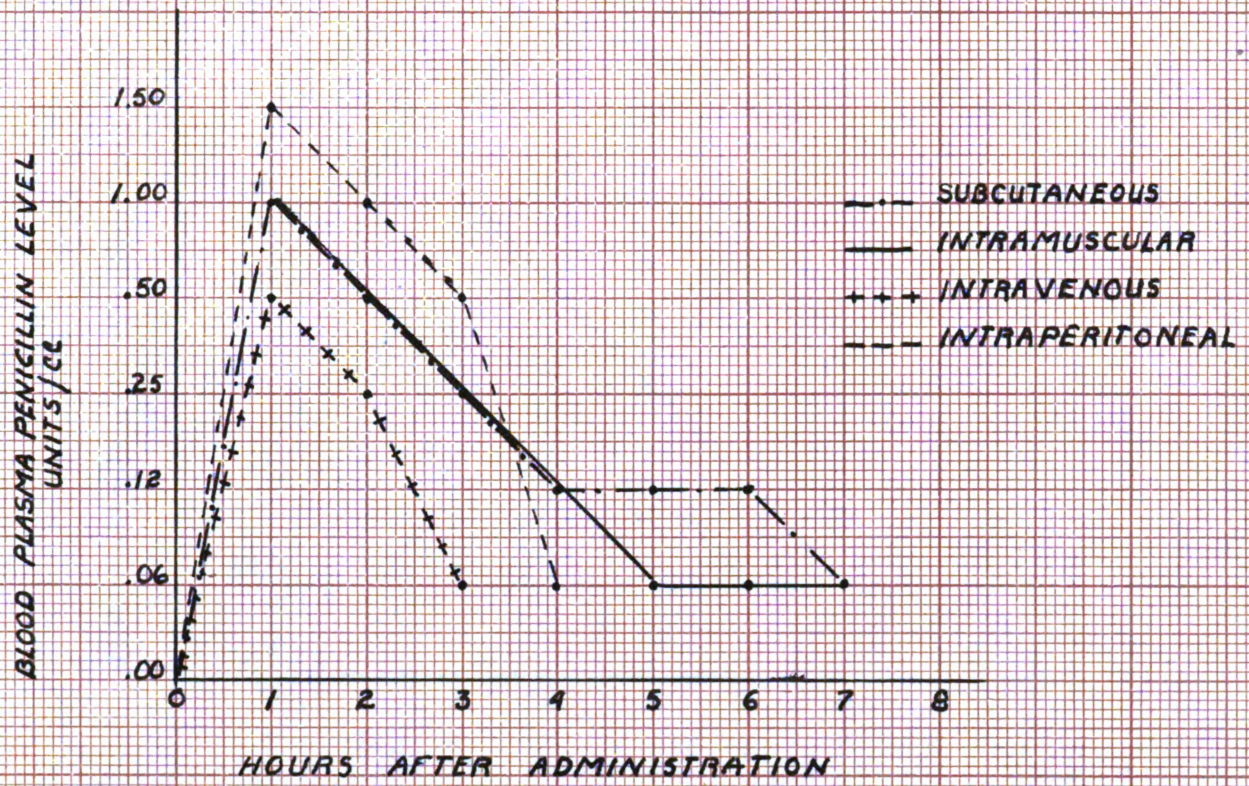
Hours After Injection	Penicillin U/cc. Plasma	W.B.C.	R.B.C.	Hb. g./100 cc.	Differential E.	B.	N.	L.	M.
1	1.0	8,400	6,300,000	12.2	7	-	21	63	9
2	1.0								
3	0.5								
4	0.25								
5	0.12	8,200	7,270,000	12.4	9	-	24	59	8
6	< 0.06								

Graph 1.
The blood plasma levels obtained in the cow when 300 units per lb. of body weight of crystalline penicillin G was administered by the subcutaneous, intramuscular, intravenous and intraperitoneal routes.

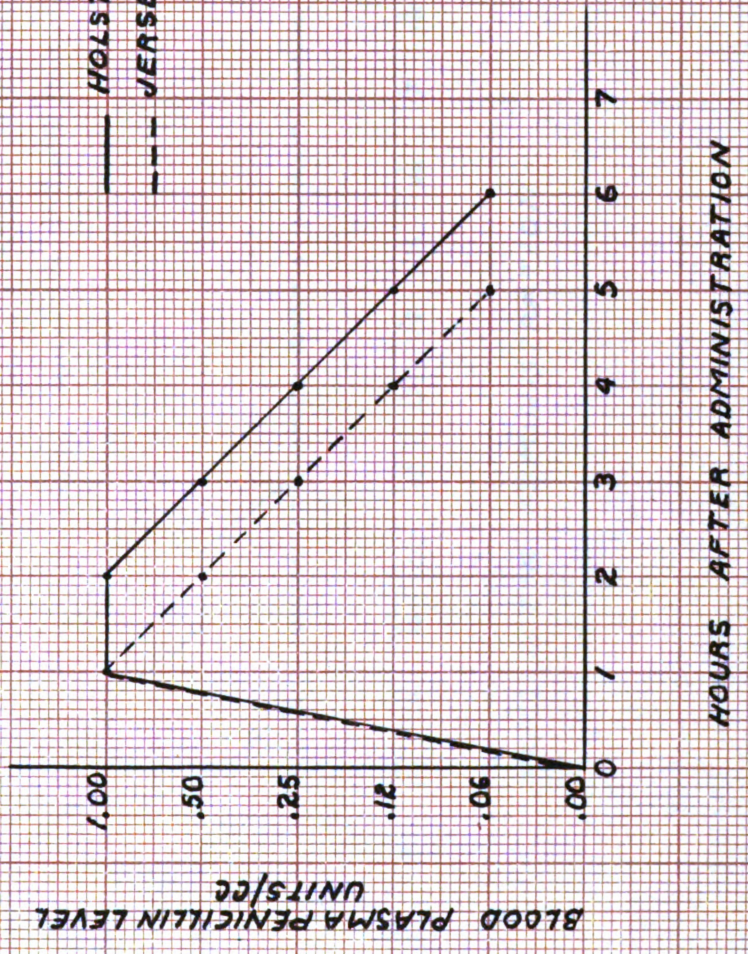


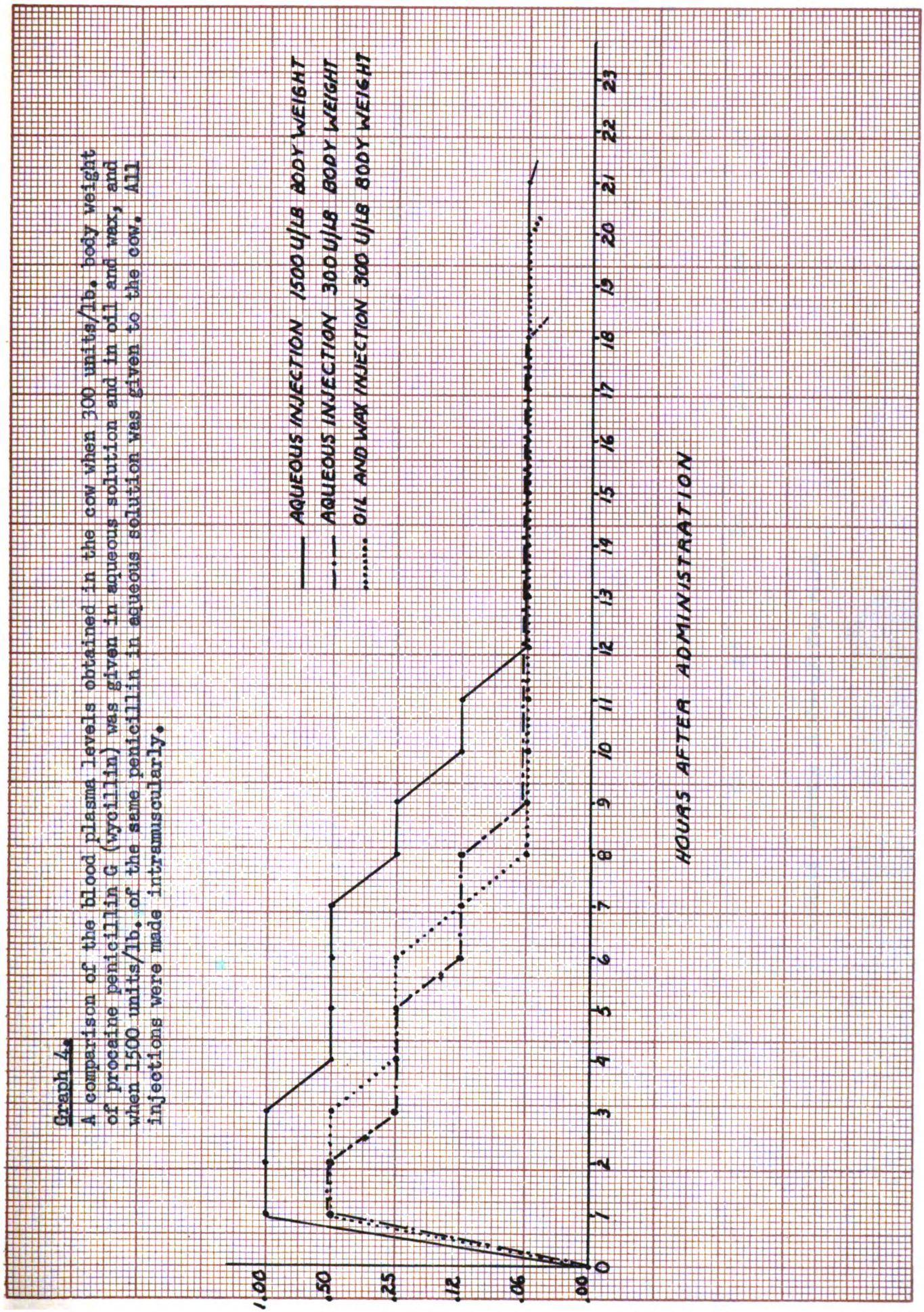
Graph 2

The blood plasma levels obtained in the cow when 500 units per lb. of body weight of crystalline penicillin was administered by the subcutaneous, intramuscularly, intravenously and intraperitoneal routes.



Graph 3.
Penicillin blood plasma levels obtained in the cow when single injections of Romansky's formula was administered subcutaneously at a rate of 200 units per lb. body weight.

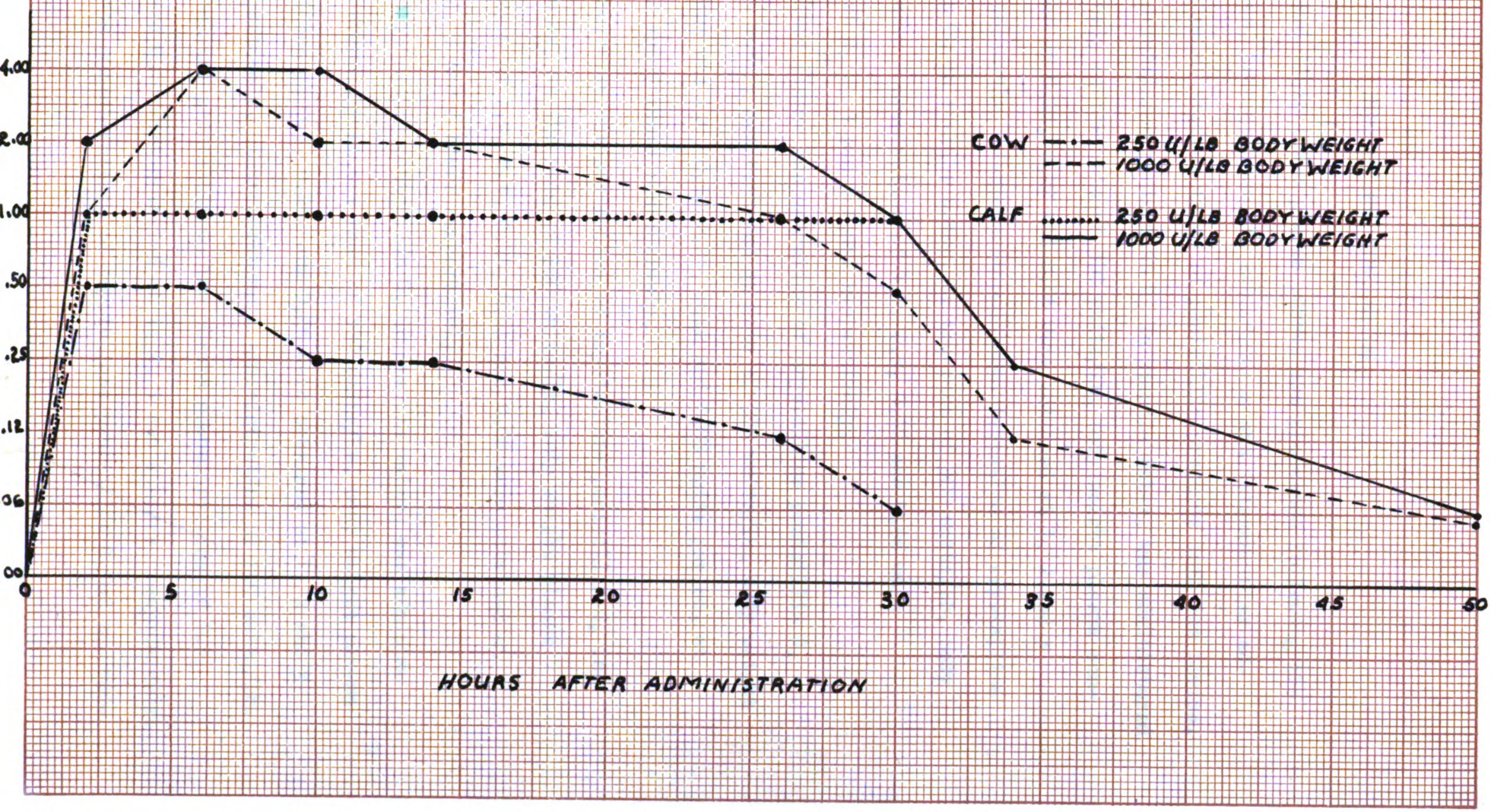




Graph 4.
A comparison of the blood plasma levels obtained in the cow when 300 units/lb. body weight of procaine penicillin G (wycillin) was given in aqueous solution and in oil and wax, and when 1500 units/lb. of the same penicillin in aqueous solution was given to the cow. All injections were made intramuscularly.

Graph 5.

The blood plasma level of penicillin when S-R penicillin was administered intramuscularly to a cow and a calf at the rate of 250 units/lb. body weight and 1000 units/lb. body weight.



SUMMARY AND CONCLUSION

The penicillin blood plasma curves are presented following the intravenous, intraperitoneal, subcutaneous and intramuscular administration of various types of penicillin products into the bovine.

When crystalline penicillin G, in sterile water, was administered intravenously at the rate of 300 units/lb. body weight plasma concentrations of 0.25, 0.12 and 0.06 were obtained at 1, 2 and 3 hours respectively. Intraperitoneal administration of crystalline penicillin G, in sterile water, at the rate of 300 units/lb. body weight plasma concentration of 1.0, 0.5, 0.12 and 0.06 were obtained at 1, 2, 3 and 4 hours respectively.

The blood plasma concentration of crystalline penicillin G, in sterile water, administered at rate of 300 units/lb. body weight subcutaneous was 1,0, 0.5, 0.25, 0.12 and 0.06 units per cc. at 1, 2, 3, 4 and 5 hours respectively.

Intramuscular administration of crystalline penicillin G, in sterile water, at the rate of 300 units/lb. body weight plasma concentration of 1.0, 0.5, 0.25, 0.12 and 0.06 were obtained at 1, 2, 3, 4, 5 and 6 hours respectively.

When crystalline penicillin G, in sterile water, was administered intravenously at the rate of 500 units/lb. body weight plasma concentrations of 0.5, 0.25, and 0.06 were obtained at 1, 2 and 3 hours respectively. Intraperitoneal administration of 500 units/lb. body crystalline penicillin G, the plasma concentrations of 1.5, 1.0, 0.5 and 0.06 were obtained at 1, 2, 3 and 4 hours, respectively. Subcutaneous administration of crystalline penicillin G, at the rate

of 500 units/lb. body weight plasma concentrations of 1.0, 0.5, 0.25, 0.12, 0.12, 0.12 and 0.06 were obtained at 1, 2, 3, 4, 5, 6 and 7 hours respectively.

Intramuscular administration of crystalline penicillin G, in sterile water, at the rate of 500 units/lb. body weight plasma concentrations of 1.0, 0.5, 0.25, 0.12, .06, .06 and .06 were obtained at 1, 2, 3, 4, 5, 6, and 7 hours respectively.

When Romansky's formula administered subcutaneously at rate of 300 units/lb. body weight plasma concentrations of 1.0, 1.0, 0.5, 0.25, 0.12 and 0.06 were obtained at 1, 2, 3, 4, 5 and 6 hours respectively. When procaine penicillin G, aqueous solution, was administered subcutaneously at the rate of 1500 units/lb. body weight plasma concentrations of 1.0 unit for 3 hours, 0.5 unit for 4 hours, 0.25 for 2 hours, 0.12 for 2 hours, and 0.06 from 12 hours through 21 hours. An aqueous solution and oil and wax administered at rate of 300 units/lb. body weight plasma concentrations of 0.5 units for 2 hours, decline to 0.25 for 3 hours, 0.12 units for 3 hours and finally to 0.06 for the remainder of 18 hours respectively.

During various series of penicillin administration procaine S-R was injected at rate of 250 and 1000 units/lb. body weight to cow and calf. The blood plasma concentrations of 250 units/lb. body weight for calf yielded a plasma level of 1.0 unit for 30 hours. In the cow this same dosage was somewhat lower, 0.5 for 10 hours, .15 units for 15 hours and 0.06 for the remaining 5 hours.

In the calf when the rate was given at 1000 units/lb. body weight the blood plasma concentrations was 2.0, 4.0, 4.0, 2.0, 2.0, 1.0 0.25 and 0.06 were obtained at 2, 6, 10, 14, 16, 26, 30 and 50 hours respectively.

Subcutaneous injections of 1000 units/lb. body weight of S-R plasma concentrations of 1.0, 4.0, 2.0, 2.0, 1.0, 0.5, 0.25 and 0.06 were obtained at 2, 6, 10, 14, 26, 30, and 50 hours respectively.

No local or systemic reactions were rated following the injections of various penicillin preparation used in these experiments. Studies of the blood picture indicate the single injections of penicillin in dosage of 250, 300, 500 and 1000 units per pound of body weight had no immediate effect on altering the red and white cell counts, differentials count, or hemoglobin content in the normal healthy cow.

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