BLOOD STUDIES IN DOGS FOLLOWING THE INJECTION OF PENICILLIN

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

W. O. Brinker

1947

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BLOOD STUDIES IN DOGS

FOLLOWING THE INJECTION OF PENICILLIN

BLOOD STUDIES IN DOGS FOLLOWING THE INJECTION OF PENICILLIN

by

W. O. BRINKER

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

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INTRODUCTION

One of the major problems confronting the veterinary practitioner today is the paucity of basic knowledge underlying the use of drugs. With the advent of newer antibiotics, which are more specific in action, and more specific in methods of administration this defect becomes increasingly apparent. If these agents are to be used to the best advantage in the treatment of infections caused by susceptible organisms, basic studies are essential.

The following study was limited to certain observations on penicillin in dogs. The two main objectives upon which this investigation was based were: (1) The determination of penicillin blood plasma levels in dogs following the administration of this agent in various vehicles and by the different routes of injection. (2) A consideration of the immediate effect of penicillin on the blood picture following single injections.

REVIEW OF LITERATURE

The results obtained by the use of vehicles to retard absorption of penicillin compared with those obtained by dissolving it in physiological saline have been reported by various authors.

Armstrong, Halpern, and Cutting (1945) after experimental work on doga using various vehicles concluded that none were more effective than 5 per cent dextrose alone in sustaining penicillin levels in the blood sera. The various vehicles which they used included methyl cellulose, human plasma, 12 per cent sucrose, 12 per cent lactose, sesame or peanut oil, 4.5 to 25 per cent sorbitol, 8.5 per cent gelatin and urea, congo red and dextrose, epinephrine and gelatin, and pitressin and dextrose. In using 5 per cent dextrose as a vehicle in human patients, they found that with smaller doses of penicillin the increased level could hardly be called important, but with larger doses it was great enough to be theoretically valuable. With doses of 40,000 units and 80,000 units, the measurable serum level was maintained for one hour longer than a similar dose given in physiological saline.

Fish, Foord and Allis (1945) compared the serum levels obtained in rabbits after injections of penicillin in physiological saline with those obtained from the injection of penicillin in physiological saline and adrena-

line. Using saline as a vehicle, measurable levels were maintained for approximately 1.5 hours, whereas, saline plus adrenaline maintained the measurable level for 3 to 4 hours. The addition of adrenaline was observed to level off the peak and increase the duration of the blood concentration curve. Similar results were obtained in human patients. 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.

Parkins, Wiley, Chendy and Zintel (1945) report that the use of colloidal 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. They comment that the colloidal gelatin delayed absorption and extended the effect of the vasoconstrictor as well as that of the penicillin, and the vasoconstrictor, in turn, delayed the absorption of the gleatin acting as a vehicle for penicillin. 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.

The efficacy of various agents for delaying absorption of penicillin in the horse was reported by Doll and Dimock (1946). 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.

A simple rapid technique for preparing water-in-oil emulsions of penicillin was reported by Freund and Thompson (1945). They prepared the emulsion by first injecting 1.4 cc. of sterile physiological saline into a sterile vial containing 100,000 units of penicillin. To this solution was added 3.1 cc. of a lanolin-peanut oil mixture. This addition was made with a sterile syringe adapted with a seventeen gauge needle. Emulsification was accomplished by repeated with-drawals and injections of the penicillin-oil mixture from the syringe into the vial containing the original penicillin.

Cohn and others (1945) using the emulsion technique reported measurable serum levels in man at four hours in comparison to measurable levels at three hours following a single injection of 150,000 units in aqueous solution. They also reported that giving part of the total unitage in aqueous solution and part in water-in-oil emulsion sustained the level somewhat longer. This appears to be substantiated in practical clinical tests in the treatment of gonorrhea as Cohn, Kornblith, Crumstein, Freund and Thompson (1946) found that one injection of 150,000 units of sodium penicillin in a

water-in-oil emulsion effected a cure of 101 patients in a series of 105; also that the simultaneous injection of 100,000 units in water-in-oil and 50,000 units in aqueous solution cured all 49 patients treated.

The results reported by Ory, Wilcox and Finland (1946) on comparative serum levels following single intramuscular injections of penicillin in saline and penicillin in water-in-oil emulsion did not substantiate those of Cohn and others in that the latter did not sustain the serum level longer. When tested in the same subject, they found the serum concentrations obtained after a single injection of penicillin in a water-in-oil emulsion were not superior to those obtained with the same dose given in the same volume of saline, and the levels were not better sustained. Their findings were based on single intramuscular injections of 100,000, 200,000 and 300,000 units.

Horus, Wilcox and Finland (1946) reported on a second series of observations on comparative penicillin serum levels. This time "emulgan" which is composed of sesame oil and cholestrin base was compared with saline as a vehicle. When tested on the same patient, saline compared very favorably with the water-in-oil emulsion as a vehicle in sustaining the penicillin serum level.

Doll and Hull (1947) 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 4 hours and water-in-oil emulsion maintained the level 4 to 5 hours.

Jones, Donaldson and Allen (1946) found that the use of magnesium sulfate monohydrate with sodium penicillin and peanut oil delayed the absorption of penicillin. They reported finding 0.03 units per cc. of serum in eight of eleven patients twenty-four hours after a single subcutaneous injection of 250,000 units in this vehicle. Clinical results showed 108 of 113 cases of gonorrhea (95.6 per cent) "cured" using this method.

Penicillin was suspended in a dry sterile vegetable oil (peanut oil) by Raiziss (1944). The blood of rabbits was found to be bacteriostatic against Staphylococcus aureus for a longer period of time following an intramuscular injection of penicillin in the oil suspension when compared with a similar injection in aqueous solution. He also found that penicillin in oil was therapeutically more effective in the treatment of experimental rabbit syphilis than were aqueous solutions of penicillin.

Favorable therapeutic serum concentrations (0.007 and 0.019 units per cc.) were maintained for eight hours in 25 patients after a single intramuscular injection of 150,000

units of penicillin in a beeswax (1 to 2 per cent) and sesame oil mixture by Zinnomon and Seeberg (1945).

Trumper and Hutter (1944) and later Trumper and Thompson (1945) reported that the absorption of penicillin could be delayed by chilling the site of injection before and after the administration of penicillin, thus giving a prolonged therapeutic value using a minimum amount of penicillin. In checking forearm blood flow they found that it ranged from 0.5 cc. (per hundred cubic cc. of arm volume per minute) at a temperature of 55.4° F to a speed of 5.9 cc. at 98.6° F. By using a thermocouple, the temperature 0.5 of an inch below the skin after an ice bag was placed on the deltoid muscle was found to be reduced to 63° F. In the clinical use of penicillin, ice bags were applied to the site of injection two hours previous and twelve hours following intramuscular injection. This procedure maintained effective bacteriostatic levels for a period of 12 hours in five out of six cases.

Chow and McKee (1945) in working with crystalline penicillin found that it combines with human serum albumin to make a large penicillin albumin complex. In mouse excretion studies, the penicillin-albumin complex was found to be excreted more slowly than the sodium salt of penicillin. The authors suggested this as another means of sustaining penicillin serum levels.

In 1941 Code and Lewis and in 1942 Code and Varco found that the action of desoxycorticosterone acetate and

histamine could be markedly prolonged by administering them parenterally in beeswax mixtures. Bryson and Code (1944) reported prolonging the action of heparin as an anticoagulant when administered in beeswax.

Romansky and Rittman (1944) observing these results reported by Code and associates in the delaying action of beeswax added it to peanut oil in preparing a vehicle to delay absorption of penicillin. They prepared their penicillin beeswax-oil suspension as follows: All equipment used was sterilized at 17 pounds steam pressure for 20 minutes. The beeswax was heated and filtered through six layers of gauze, while the peanut oil was sterilized by filtering through a Seitz filter. Various amounts of beeswax were added to the peanut oil, ranging from 0.75 to 6 per cent. The vial containing the penicillin was shaken to break it into a powdery state. To the penicillin vial were next added 3 to 5 beads and the beeswax-peanut oil mixture. It was then shaken for 15 minutes to make a uniform suspension. Calcium penicillin was used as it was less hydroscopic than sodium penicillin.

Stability tests on Romansky's mixture showed no deterioration when kept under refrigeration, at room and 37° C. temperatures for 30 to 62 days.

Rabbits given single intramuscular injections of 5,000 to 10,000 units in the suspension, sustained a measurable penicillin blood level for 6 to 12 hours, while similar amounts of penicillin injected in saline resulted in a de-

tectable level for only 2 hours.

When single intramuscular injections of 41,500 to 66,400 units in the suspension was given to humans the penicillin blood level was maintained for 6 to 7 hours. None of the patients complained of local pain or irritation. In the initial clinical trials twelve patients with gonorrhea were given from 51,250 to 100,000 units in a single intramuscular injection; eleven of the twelve were pronounced cured.

In studying tissue reactions to intramuscular injections of 3 per cent beeswax-peanut oil mixture, ten hamsters were given 0.05 to 0.10 cc. (500 to 1,000 units penicillin) twice a day for five days. They were then sacrificed at the rate of one per week and tissue sections were prepared. At twenty-four hours the muscle fibers were separated by oil cysts surrounded by polymorphonuclear leucocytes: no necrosis was present. At the end of ten days, particles of beeswax were surrounded by giant cells and scattered disintegrating leukocytes. At thirty days the beeswax had completely disappeared and the cyst walls, which were composed of minute amounts of fibrous tissue and giant cells, were partially collapsed.

A number of interesting points were bought out in a study of absorption and excretion of penicillin by Kirby, Leifer, Martin, Rammelkamp and Kinsman (1945). Three-hundred and fifty-nine patients were given intramuscular and subcutaneous injections of 300,000 units in beeswax and peanut oil. These workers noted quite a wide variation in

absorption and excretion. The sojourn of assayable levels in the blood stream of different patients ranged from 4 to 28 hours. Part of the patients in whom levels were present for 16 to 28 hours showed irregularity of absorption during the second twelve hours; i.e., there were one or more occasions on which assayable levels were not present. Excretion in the urine after the first 12 hours was found to decline rapidly, however, small amounts could be detected for 72 hours in some patients. No significant cumulative evidence was found in patients given intramuscular injections of 300,000 units of the mixture daily for eight days. Only 7 per cent had assayable levels at 24 hours following the injection. They also found that results with subcutaneous administration were superior to results with intramuscular injection both in uniformity of absorption and in prolongation of blood levels. No induration, nodule formation or sterile abscesses were observed when the mixture was administered subcutaneously.

Doll and Dimock (1946) in working on horses found that single intramuscular injections of 300 to 500 units ofpenicillin per pound of body weight given in a suspension of beeswax and peanut oil maintained effective blood concentration for four to six hours. This interval was approximately double that when saline solutions were used as a vehicle.

Bohls and Cook (1945) thought it of interest to study the effect of aluminum-potassium sulfate and peni-

cillin mixtures in delaying absorption following intramuscular injection since this aluminum material was so successful in delaying the absorption of diphtheria toxoid. Local and systemic (temperature) reactions of aluminum potassium sulfate-penicillin mixtures containing 16,000 units of penicillin and 20 mg. of aluminum were studied in normal rabbits. Control animals were injected with penicillin-beeswax-peanut oil mixtures of Romansky and Rittman (1944). No significant reactions were noted except that a small nodule could be felt at the site of injection in some of the animals for several days. Blood concentrations of the animals receiving the aluminum-penicillin mixtures compared favorably with those receiving Romansky's Formula. Both mixtures maintained assayable levels for 8 hours. Human patients injected with 50,000 units of penicillin in aluminum potassium sulfate maintained a therapeutic level for from 6 to 9 hours. No local or systemic reactions were noted.

A method of estimating penicillin levels in body fluids was reported by Randall, Price and Welch (1945). This was a serial dilution method employing a strain of Bacillus subtilis as the test organism. The strain selected was equally sensitive to penicillin when compared with most strains of hemolytic streptococci. They reported that thousands of determinations had been made with satisfactory results.

Elias, Merrion and Speicher (1945) observed that some human blood sera contained a substance inhibitory to B. subtilis and streptococcus strain C 203. They pointed out that the effects of these substances may be very easily misinterpreted as penicillin activity, especially in concentrations of the order of 0.02 to 0.05 units per cc. of serum. The sera from both children and adults contained this inhibitory substance and its activity was more pronounced against B. subtilis than towards the streptococci. Sera from ailing adults showed pronounced bacteriostatic and bactericidal activity against both microorganisms.

Chandler, Price and Randall (1945) made the same observations and also found that the inhibitory substance was both variable and transitory in a given individual. Forty per cent of the human sera tested contained an anti-subtilis factor and they also pointed out that these inhibitory substances may materially affect the interpretation of the penicillin concentration of the blood samples in amounts of 0.125 units or less.

In searching for organisms to use in determining the concentration of streptomycin in body fluids Buggs et al (1946) found that 30 out of 35 sera tested from normal persons, who had received no previous medication, contained inhibitory substances against B. subtilis, and none against Staph. aureus. This inhibitory substance was present in dilutions of serum up to 1:32.

In a recent report Hoffman (1946) commented on this

inhibitory factor. In a total of 120 plasma specimens he found only one that showed inhibition in a level equivalent to 0.03 units per cc. (1:4 dilution): the remaining specimens gave negative levels.

The Committee on Medical Research and Medical Research Council (1945) jointly made known the fact that penicillin, as it is distributed commercially, is a mixture of several substances. At least four different penicillin types are known to exist. These are disignated in the United States as F, G, K and X. Chemically they differ from one another in side groups attached to a common nuclear structure.

Prior to the above report information had begun to appear indicating that several types of penicillin were present in commercial penicillin, also that these types varied significantly in their in vitro and in vivo activity against a variety of bacteria. Libby and Holmberg (1945) found that penicillins G and X appeared equally effective on a unit basis in inhibiting growth of Staph. aureus and B. subtilis. On the basis of weight penicillin G was more effective than X. On the other organisms tested penicillin X was found to be more effective than G in inhibiting growth in vitro.

Penicillins X and G were compared with respect to sensitivity of pathogenic organisms and serum levels by Ory, Wilcox and Finland (1945). An alpha hemolytic streptococcus, a gonococcus, pneumococcus, streptococcus viridans and meningococcus were found to be two to eight times more sensitive to X than to G. Staphylococci were found to

be equally sensitive to both types. Comparative penicillin serum levels on ten convalescent patients following single intramuscular injections of 20,000 units on two successive days were significantly higher and were sustained longer using penicillin X than regular penicillin. A comparison of clinical results using the two penicillins left the authors with the impression that gonococcal infection responded somewhat more rapidly and completely when penicillin X was used, while on other infections the two showed up equally effective.

Eagle (1946) using lots of crystalline penicillins F, G, K and X compared their activity in vitro against a streptococcus on a milligram basis. Their order of efficiency was X, K, G and F. Against Spirocheta pallida the order of efficiency was G, K, F and X, while with Staph. aureus the efficiency was K, G, F and X.

The pharmalogical basis for the low therapeutic activity of penicillin K compared with that of penicillins F, G and X was determined and reported by Eagle and Musselman (1946). They found that penicillin K disappeared from the blood far more rapidly than did the other penicillins, also that a relatively smaller amount was excreted in the urine. In rabbits injected with penicillin K a measurable blood level was maintained for 45 minutes; using F, G or X the level was maintained for two hours. Urinary excretion of K averaged 33 per cent; the other three averaged 74 per cent. Approximately the same results were obtained in man.

In experimental infection in white mice the ratio of activity was found to be K-9, F-50, G-100 and X-260. These therapeutic results compared with their in vitro activity suggested that it would seem desirable to standardize impure mixtures of penicillin (since these contain various amounts of the different types) for therapeutic use by some method other than their bactericidal activity in vitro.

The Committee on Medical Research, The United States Health Service, and The Food and Drug Administration (1946) jointly reported on the changing character of penicillin. They brought out the fact that commercial penicillin might vary from time to time in the relative content of the various penicillins and that in recent months some commercial penicillins have contained a substantial proportion of penicillin K. This report also indicates that penicillin K was relatively inefficient as compared to F, G and X against several infections studied. Commercial penicillin has also undergone a change in the direction of increased purity with a consequent decrease in certain impurities which may have possessed some therapeutic activity.

The results with penicillin in the treatment of early syphilis reflects this change in character as treatment of this disease has been less satisfactory since May 1944 than prior to this date. They point out that factors responsible for the apparent decrease in efficiency are under intensive study and that practical steps to meet the difficulty are in progress by industrial producers.

The administration of penicillin by various routes was studied by Rammelkamp and Keefer (1943) to determine its absorption, excretion, and distribution. Intravenous injections of penicillin were followed by an immediate rise and very rapid fall in blood serum levels in contrast to intramuscular and subcutaneous injections in which the rise was somewhat delayed, serum levels reached were not so high, but remained for a longer period of time. Intravenous injection of 10,000 units showed an immediate value of 0.625 to 1.25 units per cc. of serum. In 30 minutes the level ranged between 0.039 and 0.156 units, 0.19 units in 60 minutes and 0.019 to 0.007 units in 90 minutes. The occurrence of the last readable levels in the various patients tested was at intervals ranging from 90 to 210 minutes.

After an intramuscular injection of 10,000 units the serum level was 0.58 units at 5 minutes, 0.078 units at 30 minutes, 0.015 to 0.039 at 90 minutes. In two subjects given 10,000 units subcutaneously a high of 0.007 units was reached, disappearing at 205 to 300 minutes.

Fleming, Suchet, Young and Rowe (1944) also studied the concentration of penicillin in the blood serum of patients who had been receiving the drug in various doses intravenously, intramuscularly, or subcutaneously as single injections. Following an injection of 15,000 units intravenously they found a maximum concentration of about 4 units per cc. almost immediately, followed by a very

rapid fall so that after ten minutes it might be only 0.5 units. Thereafter the fall was slower reaching about 0.03 units in one hour. Following a similar intramuscular injection the blood concentration at two minutes was 0.12 units per cc., reaching a maximum at 6 minutes, after which the content dropped rapidly, but not as quickly as with the intravenous injection, so that after 3 hours little or no penicillin was detectable. Subcutaneous administration was found to give almost the same curve as the intramuscular route, except that the forst appearance in the blood was delayed for a minute or two. In fifteen minutes it had reached the same maximum level as with intramuscular injection, and the fall followed the same curve.

Various doses were given intramuscularly and the time of disappearance determined. Fifteen-thousand units disappeared in 2 to 3 hours, 20,000 units in 3 hours, 35,000 units in 4 hours, 50,000 units in 4 to 5 hours, 100,000 units in 5 to 6 hours.

When purified preparations of penicillins became available, Hoffman (1946) studied the blood serum levels obtained following the subcutaneous and intramuscular routes of injection. Results of a series of 206 assays revealed that absorption and elimination from the blood stream were almost identical when given byeither route of injection. The author also reported that the patients preferred the subcutaneous injection to the intramuscular

injection since less pain was produced.

Waltz and Zintel (1945) reported their findings on the transmission of penicillin to the amniotic fluid and fetal blood in the human. Twenty-five thousand units in physiological saline were injected intramuscularly to each member of the first three groups: In group I (those delivering within 60 minutes after injection of penicillin) comparatively high maternal blood levels were reached while very little of the drug was found in the cord blood or amniotic fluid. In group II (those delivering between 60 and 90 minutes) maternal levels 0.156, cord blood 0.039 to 0.078, amniotic fluid 0.039 to 0.156. In group III (those delivering between 90 and 180 minutes) maternal levels 0.178 or less. Penicillin was not found consistantly in cord blood or amniotic fluid. Group IV received penicillin by continuous intravenous drip at the rate of 10,000 units per hour. After 2 hours the maternal blood was 0.078 to 0.312 units, cord blood 0.039 to 0.078 units and amniotic fluid 0.039 to 0.078 units. After 20 hours the maternal blood, cord blood, and amniotic fluid all contained 0.156 units. No evidance of toxicity was observed either to the mother or child in any of the groups.

Some observations on the toxicity of penicillin to animals were included in Flemings (1929) first article reporting the discovery of the new antibiotic. In working with mold broth filtrates containing penicillin he found that its toxicity to animals was very low. He reported that intravenous and intraperitoneal injections into rab-

bits and mice, respectively, resulted in no more toxic reaction than the same quantity of broth. Irrigation of human conjunctiva and infected surfaces had no irritating effect. He also reported that in vitro, penicillin which completely inhibited the growth of staphylococcus in a dilution of 1 to 600 did not interfere with leukocytic function to a greater extent than did ordinary broth.

Abrahan et al (1941) studied the effect of penicillin on leukocytes at various dilutions. They found that penicillin at a dilution of 1 to 100 inactivated the leukocytes almost immediately; at 1 to 250 more than 50 per cent of the leukocytes were active for 4 hours; at 1 to 500 the preparation was indistinguishable from the control. Injections of 20 mg. of penicillin (40 to 50 units per mg. strength) caused serious embarassment but not death in mice.

Later Flory and Jennings (1942), working with a more purified product, injected mice intravenously with 20 mg. of penicillin (250 and 350 units per mg. strength) with no observable reaction.

A toxicity investigation comparing crude preparations of penicillin (60 units per mg.) with a more purified product (300 and 400 units per mg.) was made by Robinson (1943). He found that on the basis of weight a purified penicillin preparation was somewhat less toxic than crude penicillin in spite of the marked increase in antibacterial activity.

Homre, Rake, McKee and MacPhillamy (1943) found that guinea pigs tolerated less penicillin units per kilogram of body weight when fatal doses were administered than rabbits or mice. On autopsy the guinea pigs exhibited no gross or microscopic lesions in the central nervous system, lungs, spleen, lymph nodes, intestines, pancreas, kidney, adrenal, gonads or voluntary muscles. In 20 per cent of the cases the heart showed necrosis of several muscle fibers in the left ventricle and a surrounding infiltration with monocytes and a few polymorphonuclear cells. On gross examination the site of injection was edematous and yellowish red in color. Microscopically the picture was one of subcutaneous edema and infiltration of monocytes and leukocytes. Focal areas of necrosis were present in many of the livers.

The authors also conducted experiments with mice comparing the toxicity of crude and purified preparations of penicillin and further verified the work of Abraham, Flory and Robinson in that purification lowers toxicity.

In a compiled report of 500 cases by the Committee of Chemotherapeutic and Other Agents (1943) it was concluded that the remarkable feature of penicillin was its relatively low toxicity and the extremely low incidence of reactions of a systemic nature. Material available at the time of this report was 10 to 15 per cent pure penicillin. Toxic reactions were as follows: fever - 5 cases, chills and fever - 12 cases, thrombophlebitis at site of injection

- 19 cases, urticaria - 14 cases, gluteal tenderness at site of injection - 5 cases, headaches and flushing of the face - 10 cases, tingling of testes - 2 cases, and pains in muscles -2 cases. They commented that urticaria might occur during the course of treatment or after treatment had been discontinued.

Lyons (1943) reviewed the results observed in 209 cases. He divided the reactions into two groups. (1) Reactions associated with particular batches and thought to be due to impurities were chills with or without fever after intravenous injections, eosinophilia (20 to 30 per cent), burning pains at site of intramuscular injection, headaches, faintness and flushing of face, unpleasant taste after parenteral injections, tingling of the testes, muscular cramps, and femoral phleothrombosis. (2) Reactions which were not limited to particular batches of penicillin were - urticaria 5.7 per cent, fever without urticaria, transient azotemia, and thrombophlebitis.

A summary of 100 clinical cases receiving penicillin therapy was reported by Dawson and Hobby (1944). Three patients developed mild urticaria. Chills and fever had not been observed by the authors since the early cases when the material was known to contain pyrogenic substances. Thrombophlebitis was observed in only one case. Complaints of discomfort at site of injection seemed to be connected with particular lots of material. In a majority of cases no symptoms of any nature were observed, nor had prolonged

administration led to the development of any intolerance or sensitivity.

Kolodny and Denhoff (1946) reported their observations on 124 patients. Immediate reactions following parenteral administration were shown by 16 per cent while 7 per cent showed delayed reactions. No significant relationship was found between the incidence of reactions, previous penicillin therapy, recurrence of symptoms following additional therapy, time interval between courses of penicillin or past history of personal or familial allergies. Relief by epinephrine or ephedrine was definite.

Hypersensitivity of the tuberculin type was reported by Welch and Rostenberg (1944) following intradermal injections of penicillin. The authors later (1945) tested 144 persons and found that 5.5 per cent exhibited this type of sensitivity. It did not occur following intramuscular or subcutaneous injections as the penicillin was absorbed and excreted too rapidly.

An investigation was carried out by Herwick, Welch, Putman and Gamboa (1945) to determine why certain lots of commercial penicillin produced severe pain on intramuscular injection. They found no correlation between the irritation produced by intradermal injections of penicillin in man or rabbits and that produced by intramuscular injections of this material in 100 patients. They also found, by using the blindfold test on 100 patients, that intramuscular injections of penicillin produced a greater incidence and intensity of pain than did the injection of isotonic salt sol-

ution. A significant correlation between purity (units per mg.) and irritation following intramuscular injection was found. An increase in potency was associated with a corresponding decrease in pain produced.

Using purified preparations of penicillin, Hoffman (1946) reported that there were no recognized cases of urticaria or other allergic manifestations. He suggested that these findings might be related to the purity of the preparation used.

Five patients who developed urticaria associated with penicillin therapy were studied for hypersensitivity and for circulating antibodies by Colloway and Barefoot (1946). They found all five to be negative when subjected to intracutaneous or passive transfer tests. Precipitation tests were inconclusive as all patients, including controls, showed a fine precipitate. The authors assumed that an excessive amount of histamine was present as in all instances the urticaria was controlled by Benadryl.

The effect of prolonged administration of penicillin upon the blood picture of canines was studied by Bailey (1946). Examinations made included coagulation time, erythrocyte counts, leukocyte counts, differential leukocyte counts, hemoglobin determinations and erythrocyte sedimentation rates, bleeding time and percentage of packed blood cells. The only two changes observed were an increase in eosinophiles and a decrease in the erythrocyte sedimentation rate.

Malkamus and Oppermann (1944) reported the normal ranges of blood constituents in the dog as follows: erythrocytes 5.5 to 8 million per cu. mm., leukocytes 9 to 10 thousand per cu. mm., and the leukocyte differential count was 2 to 4 per cent eosinophiles, 0 to 0.5 per cent basophiles, 13 to 32 per cent lymphocytes, 3 to 5 per cent monocytes.

The normal ranges of the canine blood constituents reported by Coffin (1945) were as follows: erythrocytes 5.5 to 8.8 millions per cu. mm., leukocytes 5 to 20 thousand per cu. mm., hemoglobin 12.5 to 17.3 g. per 100 cc., neutrophiles 3.6 to 15 thousand per cu. mm., eosinophiles 0.1 to 2 thousand per cu. mm., basophiles 0 to 0.4 thousand per cu. mm., lymphocytes 0.6 to 6 thousand per cu. mm., monocytes 0.1 to 2.4 thousand per cu. mm.

Boddie (1946) reported the following as normal ranges in dogs: erythrocytes 5.1 to 7.6 million per cu. mm., leukocytes 8 to 14.6 thousand per cu. mm., hemoglobin 11.2 to 14.8 g. per 100 cc., and the leukocyte differential count was 0 to 4 per cent eosinophiles, 67 to 81 per cent neutrophiles, 14.6 to 25.4 per cent lymphocytes, 1 to 7 per cent monocytes.

METHODS

The procedures used in these experiments were as follows:

Estimation of Penicillin in Body Fluids. The official test of the Food and Drug Administration, developed by Randall, Price and Welch (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 and serial dilutionsby halves made by adding one-half cc. of the fluid being tested to one of the tubes and carrying one-half cc. in serial dilution for as many tubes as necessary (usually 5 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 by halves. 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 over night (18 hours). The last tube in which no growhh occurs is taken as the end-point. This was usually sharp, inasmuch as one tube was clear while

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

the next one in the 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. Ordinarily the test as described here is 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 (Sanford et al, 1933). 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 such as a 50 cc. Erlenmeyer flask. To this was added 0.1 cc. of blood. The mixture was thoroughly shaken and then transfered to the photelometer for reading using a green filter. This reading was then obtained in grams of hemoglobin per 100 cc. of blood from a calculated table.

Leukocyte and Erythrocyte Enumeration. Leukocyte and erythrocyte counts were made within 24 hours after bleeding using Thoma diluting pipettes and Bright-Line Improved Neubauer Counting Chambers (Coffin, 1945). Two per cent oxalic acid (Jones, 1927) was used as the diluting fluid for counting leukocytes, while Leak and Guy diluting fluid (Todd, 1943) was used for making erythrocyte counts.

Differential Leukocyte Counts. Blood smears were pre-

pared within one hour after bleeding by the two-slide method and stained with Wright's stain (Coffin, 1945) immediately following drying.

The dogs used in this study were obtained from a city dog pound. On arrival from the pound each individual was given 40 cc. of an homologous anti-canine distemper serum subcutaneously. In ten days an additional 40 cc. of serum was given to each dog. None of the dogs developed distemper and all remained free of the disease throughout the course of study. They were checked routinely for internal and external parasites and then treated when necessary. Their ages ranged from approximately four months to ten years. The animals were fed once each day; the ration consisted of one part of cooked meat scraps and two parts of commercial cereal dog food. This was fed at approximately six p.m. Water was kept before the dogs at all times. Penicillin injections and drawing of blood samples were always begun in the morning or at noon, depending on the number of samples collected. All the animals gained weight steadily during the entire course of study, the individual gains ranged from 10 to 25 pounds. At the termination, the dogs were autopsied and examined for lesions, particularly at the sites of injection.

The anticoagulant used in collecting blood samples was 7.5 per cent potassium oxalate; two drops* of this solution

*A commercial eye dropper was used. The delivery end had a diameter equal to the No. 16 opening on U. S. Standard Gauge for Sheet and Plate Iron and Steel N. 283.

was used to each 5 cc. of blood.

RESULTS AND DISCUSSION

The problem of immediate importance was to determine if the amount of anticoagulant used (potassium oxalate) had any effect on inhibiting the growth of B. subtilis, the test organism. It was also thought to be of interest to determine the amount necessary to stop the growth of the organism. This was accomplished in the following manner: Five series of ten tubes each were set up. To each was added 0.5 cc. of B. subtilis inoculated yeast beef broth. Serial dilutions were then prepared using 0.5 cc. amounts of aqueous solutions containing various quantities of 7.5 per cent potassium oxalate. The results of this experiment are presented in Table I.

Table I. BACTERIOSTATIC EFFECT OF POTASSIUM OXALATE ON B. SUBTILIS.

Amount of Potassium Oxalate Added	Tubes 1	2	3	4	5	6	7	8	9	10
1 drop	P	P	P	P	P	P	P	P	P	P
2 drops	P	P	P	P	P	P	P	P	P	P
5 drops	P	P	P	P	P	P	P	P	P	P
10 drops	-	P	P	P	P	P	P	P	P	P

\- no growth
P growth with pellicle formation

It was found that approximately an equal amount of anticoagulant, 7.5 per cent potassium oxalate, could be added before inhibiting the growth of B. subtilis. Since only two drops were added per 5 cc. of blood in drawing blood samples, its inhibitory action was considered insignificant.

Another problem of immediate importance was to determine if the blood of any of the dogs had an inhibitory factor against B. Subtilis as this would influence the interpretation of results. An inhibitory factor in human blood sera was found to be quite variable. Chandler, Price and Randall (1945) reported that 40 per cent of the patients examined carried this factor, Buggs et al (1946) reported 85.7 per cent and Hoffman (1946) reported less than 1 per cent. The possible significance of this factor in the present study was determined in the following manner: A series of 4 tubes was set up for each dog. One-half cc. of yeast beef broth was added to the third and fourth tube of each series. One cc. of serum was added to the first tube, 0.5 cc. to the second tube, 0.5 cc. to the third tube and serially diluted through the fourth tube. One and one-half cc. of a 1:100 dilution of the test organism in yeast beef broth was added to all tubes; then incubated at 37° C for 18 hours. The blood plasma of all dogs used in this experiment was checked at different intervals throughout the course of study using the above procedure. The results of the initial, midway and final check are shown in Table II. Tube 1 represents a serum

dilution of 1:2.5, tube 2 a dilution of 1:4, tube 3 a dilution of 1:8 and tube 4 a dilution of 1:16.

Table II. EVIDENCE OF INHIBITORY FACTOR IN DOG SERA AGAINST B. SUBTILIS.

Dog No.	Feb./46 Test Tubes				June/46 Test Tubes				Jan./47 Test Tubes			
	1	2	3	4	1	2	3	4	1	2	3	4
100	G	P	P	P	-	P	P	P	P	P	P	P
200	P	P	P	P	-	P	P	P	P	P	P	P
300	P	P	P	P	G	P	P	P	P	P	P	P
400	G	P	P	P	-	G	P	P	G	G	P	P
500	G	P	P	P	-	P	P	P	P	P	P	P
600	G	P	P	P	G	G	P	P	G	P	P	P
700	G	G	P	P	G	G	P	P				
800	G	P	P	P	G	G	P	P	P	P	P	P
900	G	P	P	P	-	P	P	P	P	P	P	P
000	G	G	P	P	-	P	P	P	G	G	P	P
L00	G	P	P	P	G	G	P	P	G	P	P	P
B00	G	P	P	P	-	G	P	P	G	G	P	P

P growth with typical pellicle formation
G flocculent growth without pellicle formation
- no growth

These data agree with that reported by Chandler, Price and Randall (1945) in that the inhibitory substance was both variable and transitory in a given individual. In all the dogs the 1:4 dilution of serum showed growth, either typical pellicle formation or flocculent type. In the actual testing for the blood serum concentration of penicillin a sufficient number of hourly blood samples were drawn so that in every case the study was carried forward far enough to show growth in the 1:4 dilution tube. Thus each series of samples on an individual acted as a check on the presence or absence of the inhibitory factor even though a sample was not drawn specifically for this purpose prior to injecting the penicillin. The number of animals used in this experiment was too small to draw any conclusions, however, this group of dogs at no time carried the inhibitory factor in sufficient concentration to interfere with the method used for estimating penicillin levels in body fluids.

The first objective in this study was to compare penicillin blood plasma levels following a single injection of a fixed unitage of penicillin given by the different routes of administration, namely: intravenously, intraperitoneally, intramuscularly, and subcutaneously. One-thousand units per pound of body weight was decided upon as the trial dosage.

Five per cent dextrose was used as the vehicle since Armstrong, Halpern and Cutting (1945) concluded from their

experiments that it was equally efficient or more so than other vehicles tested in sustaining penicillin levels in the blood sera. The test solution was made up so that each cc. contained 10,000 units of penicillin.

In the series of trials using the intravenous route the penicillin was injected into the left radial vein. In 5 minutes the first blood sample (5 to 7 cc.) was drawn from the right radial vein. Blood samples were then taken at half-hour intervals for the next three hours. Beginning with the first sample drawn, blood was taken at hourly intervals and checked for red and white cell counts, hemoglobin content, and leukocyte differential counts, to determine if penicillin had any effect on altering these in the normal healthy animal.

In the series of trials using the intraperitoneal, intramuscular, and subcutaneous routes blood samples were drawn one-half hour following the injection, and then at hourly intervals. In these three series of studies samples were taken at the beginning and end of each individual trial to determine the effects of penicillin on the blood picture.

Intraperitoneal injections were made in the lateral abdominal region. The subcutaneous injections were made in the nuchal region approximately on the midline. This location was chosen as it is readily accessible and caused least discomfort to the patient, particularly when a 24

gauge needle was used. Intramuscular injections were made in the posterior portion of the external femoral region about midway between the stifle and hip joint. The needle was inserted deep into the muscle, usually about 3/4 of an inch.

Four dogswwere used in the series injected intravenously - 601, 201, 101 and 701; four in the intraperitoneal - 103, 203, 301 and 403; eight in the subcutaneous - 901, 001, 502, B02, 801, 002, 902 and B03; and four in the intramuscular - L01, B01, 102 and 202. Tables III to XXII inclusive.

As the penicillin was introduced directly into the circulatory system when given intravenously it was found almost immediately at a concentration of 4 to 8 units per cc. of blood plasma. The concentration was found to fall quite rapidly so that after 30 minutes it ranged between 0.5 and 1 unit in the four dogs tested. From this point on the drop was more gradual, disappearing below measurable levels in 2 to 2.5 hours.

The intraperitoneal, subcutaneous and intramuscular penicillin blood plasma levels compared very closely with each other both in regard to the concentration reached at 30 minutes, and to the gradual drop in concentration from this point until the penicillin was no longer measurable, which in each case was between 3.5 and 4.5 hours.

The highest concentration at 30 minutes was shown by the intraperitoneal route; this ranged between 0.5 and

2 units. The subcutaneous was lowest, ranging between 0.125 and 1 unit, and the intramuscular ranged between 0.5 and 1 unit. At 1.5 hours the intraperitoneal and intramuscular levels ranged between 0.06 and 0.5 units, while the subcutaneous ranged between 0.125 and 0.5 units. At 2.5 hours the intraperitoneal route levels ranged at 0.03 and 0.25 units, while the subcutaneous and intramuscular held at 0.03 to 0.125 units. The measurable levels of penicillin on all three routes disappeared between 3.5 and 4.5 hours, however, seven out of eight dogs still had a level of 0.015 units or higher at 3.5 hours when penicillin was administered by the subcutaneous route while two out of four sustained this level by the intraperitoneal route and three out of four by the intramuscular route.

Graph 1 shows the average penicillin blood plasma level curves for each of the four routes of injection. When these curves on canines were compared with curves on human penicillin blood plasma levels (Fleming et al, 1944, and Hoffman, 1946) following single injections of penicillin made by different routes of injection, they were found to be quite similar.

From a clinical standpoint the primary interest was (1) to determine the route which was most convenient for administration and (2) to determine the route or routes which were equally effective or advantageous in maintaining effective penicillin plasma levels.

Table III Determinations on Dog No. 601 2/21/46

Route - Intravenous Female 35 lbs. 9 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy except that the dog had exhibited occasional symptoms of muscular pain.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
5 min.	4.	17,800	7,190,000	14.5	2		75	15	8
.5 hr.	1.								
1.0 hr.	.125	16,600	7,000,000	14.6	1	2	79	13	5
1.5 hr.	.06								
2.0 hr.	.03	16,900	7,200,000	14.7	3		79	12	6
2.5 hr.	<.015								
3.0 hr.	<.015	16,200	6,920,000	14.2	4		78	12	6

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

Table IV Determinations on Dog No. 201 2/21/46

Route - Intravenous Male 40 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
5 min.	4.	18,550	6,450,000	16.9	2		85	9	4
.5 hr.	.5								
1.0 hr.	.125	18,500	6,500,000	16.7	4		83	8	5
1.5 hr.	.03								
2.0 hr.	<.015	17,000	5,560,000	16.5	2		78	11	9
2.5 hr.	<.015								
3.0 hr.	<.015	16,950	5,690,000	16.2	3		81	11	5

Table V Determinations on Dog No. 101 3/11/46

Route - Intravenous Male 30 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
5 min.	4.	18,750	7,130,000	16.8	3		76	13	8
.5 hr.	1.								
1.0 hr.	.125	17,400	7,370,000	16.8	5		77	10	8
1.5 hr.	.06								
2.0 hr.	.015	17,800	6,900,000	16.2	2		81	13	4
2.5 hr.	<.015								
3.0 hr.	<.015	18,100	6,610,000	16.2	2		75	15	8

Table VI Determinations on Dog No. 701 2/21/46

Route - Intravenous Male 34 lbs. 1½ yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy except for several small areas of demodectic mange.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
5 min.	8.	15,900	4,790,000	14.4	4		75	11	10
.5 hr.	1.								
1.0 hr.	.25	15,850	5,000,000	14.1	5		80	10	5
1.5 hr.	.06								
2.0 hr.	.015	16,000	5,180,000	13.8	2		76	12	10
2.5 hr.	< .015								
3.0 hr.	< .015	15,900	4,980,000	13.6	3		73	15	9

Table VII Determinations on Dog No. 103 6/8/46

Route - Intraperitoneal Male 35 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	17,500	6,970,000	16.2	8		76	10	6
1.5 hr.	.06								
2.5 hr.	.06								
3.5 hr.	<.015								
4.5 hr.	<.015	16,600	7,200,000	15.8	5		70	15	10

Table VIII — Determinations on Dog No. 203 — 6/8/46

Route - Intraperitoneal — Male — 52 lbs. — 2 yrs.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	15,300	6,000,000	15.4	6		70	20	4
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	<.015								
4.5 hr.	<.015	14,600	5,910,000	15.9	3		62	23	12

Table IX Determinations on Dog No. 301 5/25/46

Route - Intraperitoneal Male 32 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	2.	12,450	6,070,000	17.2	4		69	21	6
1.5 hr.	.25								
2.5 hr.	.25								
3.5 hr.	.03								
4.5 hr.	<.015	10,950	6,050,000	17.8	3		66	24	7

Table X — Determinations on Dog No. 403 — 5/25/46

Route - Intraperitoneal — Male — 55 lbs. — 4 yrs.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	13,250	6,940,000	16.9	4		70	17	9
1.5 hr.	.5								
2.5 hr.	.03								
3.5 hr.	.03								
4.5 hr.	<.015	14,050	6,210,000	17.2	3		66	18	13

Table XI Determinations on Dog No. B03 6/13/46

Route - Subcutaneous Female 40 lbs. 4 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History- Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	10,100	6,400,000	15.4	6		64	20	10
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	.015								
4.5 hr.	<.015	10,250	6,350,000	14.5	5		66	14	15

Table XII Determinations on Dog 902 6/13/46

Route - Subcutaneous Female 31½ lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy, but thin in flesh. Sedimentation rate on blood samples was noticed to be very rapid.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	1.	4,900	5,540,000	12.8	3		61	21	15
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.015								
4.5 hr.	<.015	5,300	5,900,000	12.8	6		66	18	10

Table XIII — Determinations on Dog No. 002 — 6/13/46

Route - Subcutaneous — Female — 40 lbs. — 9 mo.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	13,350	6,510,000	16.2	2		65	21	12
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.06								
4.5 hr.	<.015	13,350	6,510,000	15.7	5		60	23	12

Table XIV Determinations on Dog No. 801 6/13/46

Route - Subcutaneous Male 44 lbs. 9 Mo.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	17,600	6,950,000	14.4	4		70	13	13
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	< .015								
4.5 hr.	< .015	17,800	6,860,000	15.	6		72	9	13

Table XV — Determinations on Dog No. 802 — 6/6/46

Route - Subcutaneous — Male — 38 lbs. — 4 Yrs.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	12,800	5,980,000	14.5	4		64	20	12
1.5 hr.	.25								
2.5 hr.	.125								
3.5 hr.	.06								
4.5 hr.	<.015	12,400	5,890,000	14.9	6		63	18	13

Table XVI — Determinations on Dog No. 502 — 6/6/46

Route - Subcutaneous — Male — 36 lbs. — 9 yrs.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.25	13,850	5,900,000	13.7	5		66	17	12
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	.015								
4.5 hr.	<.007	11,500	6,260,000	13.4	3		64	18	15

Table XVII — Determinations on Dog No. 001 — 5/22/46

Route - Subcutaneous — Male — 42 lbs. — 8 mo.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	14,500	6,480,000	15.7	2		72	18	8
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	.015								
4.5 hr.	<.015	13,900	6,210,000	16.5	5		71	16	8

Table XVIII Determinations on Dog No. 901 5/22/46

Route - Subcutaneous Male 30 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - 5%dextrose in phys. saline.

History - Apparently healthy except for an area of eczema (2" in diameter) on the right side of the head. Animal had been found to be positive for Leptospirosis on a number of examinations during the previous 5 mo. (Dark-field exam.)

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.125	26,700	5,430,000	14.9	6		79	8	7
1.5 hr.	.125								
2.5 hr.	.03								
3.5 hr.	.015								
4.5 hr.	< .015	25,000	6,200,000	15.2	4		79	11	6

Table XIX Determinations on Dog No. L01 5/16/46

Route - Intramuscular Female 42 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy. Nursing 2 puppies (2 weeks old). Mammary glands of bitch appear and feel normal on palpation but give a very small quantity of milk. Five puppies have died thus far and the remaining 2 are poorly nourished.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	17,000	5,790,000	17.	3		78	14	5
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.007								
4.5 hr.	<.007	16,300	6,290,000	16.9	4		75	14	6

Table XX Determinations on Dog No. B01 5/16/46

Route - Intramuscular Female 37 lbs. 4 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy. Eight puppies were weaned 2 weeks previous.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	16,200	6,100,000	16.9	6		76	16	4
1.5 hr.	.25								
2.5 hr.	.125								
3.5 hr.	.015								
4.5 hr.	<.007	17,000	5,800,000	17.2	5		71	20	4

Table XXI Determinations on Dog No. 102 5/20/46

Route - Intramuscular Male 35 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	17,100	6,200,000	16.5	3	2	75	11	9
1.5 hr.	.06								
2.5 hr.	.03								
3.5 hr.	.015								
4.5 hr.	<.015	16,900	6,100,000	15.9	5	1	72	13	9

Table XXII Determinations on Dog No. 202 5/20/46

Route - Intramuscular Male 52 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	13,000	6,250,000	16.2	6		60	21	13
1.5 hr.	.06								
2.5 hr.	.06								
3.5 hr.	.015								
4.5 hr.	< .015	12,150	5,740,000	15.9	6		66	20	8

After analyzing the results of the four routes of injection, the subcutaneous and intramuscular routes of administration seemed to more nearly meet the clinical qualifications required. The intravenous route was temporarily discarded because of occasional difficulty in administration and primarily because of poorly sustained blood plasma levels. The intraperitoneal route, although equally effective in sustaining blood plasma levels, was considered less practical because of necessary additional restraint of the dog on administration.

The next series of experiments were to determine the penicillin blood plasma levels using single injections of vaeious dosages per pound of body weight (250, 500 and 1,000 units) and giving the penicillin either intramuscularly or subcutaneously. The vehicle used in this series was 5 per cent dextrose in physiological saline. Studies on the blood picture were continued.

In the intramuscular series the following dogs were used:

1000 units per lb. body weight - L04, 604, 305 and 107.

500 units per lb. body weight - B04, 802, 803 and 004.

250 units per lb. body weight - 503, 602, L02 and 603.

Tables XXIII to XXXIV inclusive.

In the subcutaneous series the following dogs were used:

1000 units per lb. body weight - 603, 905, 904 and 504.

500 units per lb. body weight - 405, 205, 104, 204, 302

and 404.

250 units per lb. body weight - 903, 003, 303 and 105. Tables XXV to XLVIII inclusive.

Graph II shows the average penicillin blood plasma level curves for different dosages administered by the intramuscular route of injection. Graph III shows the same for the subcutaneous route of injection.

The penicillin blood plasma level curves were found to be quite regular. The height and maintenance of the penicillin concentrations were also found to be directly related to dosage administered. When penicillin was administered by the intramuscular route of injection the 30 minute plasma level for 1000 units per pound of body weight was 2 to 4 units, 500 units resulted in a level of 2 units and 250 units in a level of 0.25 to 0.5 units. In all cases the drop in blood concentration was quite regular and gradual. With 1000 units per pound of body weight, values dropped below the measurable level at 3.5 to 4.5 hours, with 500 units at 3.5 hours and with 250 units at 2.5 to 3.5 hours.

Various dosages given by the subcuataneous route resulted in blood plasma levels which were found to correlate very closely with those obtained by the intramuscular route. At 30 minutes, 1000 units per pound of body weight resulted in a blood level of 4 units, 500 units per pound resulted in a level of 2 units and 250 units per pound resulted in a level of 0.5 to 1 unit. From this point the

GRAPH 1. AVERAGE PENICILLIN BLOOD PLASMA LEVEL CURVES FOLLOWING SINGLE INJECTIONS OF 1000 UNITS/POUND IN 5% DEXTROSE AND PHYSIOLOGICAL SALINE FOR EACH OF THE FOUR ROUTES OF ADMINISTRATION.

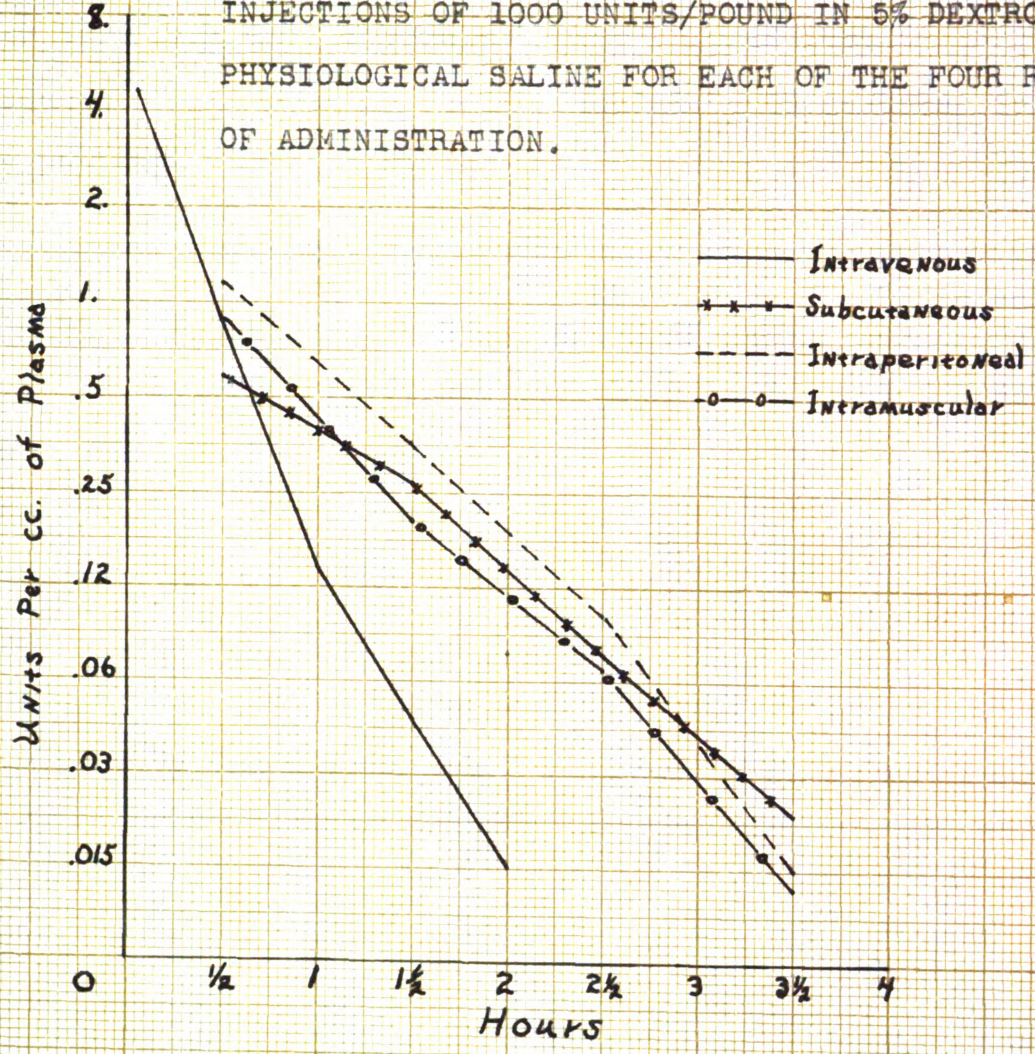


Table XXIII Determinations on Dog No. L04 11/26/46

Route - Intramuscular Female 48 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	12,300	5,690,000	15.2	3	1	66	24	6
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.06								
4.5 hr.	<.06								
5.5 hr.	<.06								
6.5 hr.	<.06	14,100	6,202,000	14.9	4	1	60	27	8

Table XXIV Determinations on Dog No. 604 11/26/46

Route - Intramuscular Male 38 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy, except for interdigital cysts on three feet.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	12,050	5,660,000	15.2	3	1	70	18	8
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	< .06								
4.5 hr.	< .06								
5.5 hr.	< .06								
6.5 hr.	< .06	13,500	5,210,000	15.5	5		69	20	6

Table XXV Determinations on Dog No. 305 11/26/46

Route - Intramuscular Male 33 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
..5 hr.	2.	13,100	6,230,000	16.	4		72	18	6
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	.06								
4.5 hr.	<.06								
5.5 hr.	<.06								
6.5 hr.	<.06	13,600	5,900,000	15.8	3		64	22	11

Table XXVI Determinations on Dog No. 107 11/26/46

Route - Intramuscular Male 38 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	14,650	6,360,000	16.5	4		74	16	6
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	< .06								
4.5 hr.	< .06								
5.5 hr.	< .06								
6.5 hr.	< .06	15,100	6,020,000	16.5	5		70	20	5

Table XXVII Determinations on Dog No. B04 7/6/46

Route - Intramuscular Female 37 lbs. 4 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	12,000	6,320,000	15.9	5		71	18	6
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	<.06								
4.5 hr.	<.06	13,300	7,010,000	16.2	4		69	18	9

Table XXVIII — Determinations on Dog No. 802 — 7/6/46

Route - Intramuscular — Male — 45 lbs. — 10 mo.

Dose - 500 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	16,200	6,010,000	14.0	2		69	18	11
1.5 hr.	.25								
2.5 hr.	.125								
3.5 hr.	<.06								
4.5 hr.	<.06	17,500	6,900,000	14.4	3		71	17	9

Table XXIX — Determinations on Dog No. 803 — 11/8/46

Route - Intramuscular — Male — 54 lbs. — 1 yr.

Dose - 500 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	14,200	6,150,000	15.4	3		72	21	4
1.5 hr.	.25								
2.5 hr.	.06								
3.5 hr.	< .03								
4.5 hr.	< .03	13,000	6,700,000	15.2	6		66	19	9

Table XXX Determinations on Dog No. 004 11/8/46

Route - Intramuscular Female 44 lbs. 1 yr.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	12,500	6,300,000	16.7	3		70	20	5
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	<.03								
4.5 hr.	<.03	13,800	6,500,000	16.4	5		66	19	8

Table XXXI — Determinations on Dog No. 503 — 8/3/46

Route - Intramuscular — Male — 33 lbs. — 9 yrs.

Dose - 250 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	12,450	5,900,000	14.5	2		64	27	7
1.5 hr.	.06								
2.5 hr.	.03								
3.5 hr.	<.03								
4.5 hr.	<.03	13,750	5,550,000	13.7	6	1	60	26	7

Table XXXII Determinations on Dog No. 602 8/3/46

Route - Intramuscular Male 37 lbs. 2½ yrs.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy except for interdigital cysts involving one front foot.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	.25	11,700	6,230,000	15.	4		60	24	12
1.5 hr.	.125								
2.5 hr.	<.03								
3.5 hr.	<.03								
4.5 hr.	<.03	12,300	6,850,000	14.9	5		68	17	10

Table XXXIII Determinations on Dog No. L02 11/8/46

Route - Intramuscular Female 49 lbs. 3 yrs.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy. This dog was kept in the Distemper Ward for the past two months.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	15,450	5,900,000	14.5	3		72	20	5
1.5 hr.	.06								
2.5 hr.	.03								
3.5 hr.	<.03								
4.5 hr.	<.03	14,750	5,550,000	14.8	6	1	60	26	7

Table XXXIV Determinations on Dog No. 603 11/8/46

Route - Intramuscular Male 37 lbs. 2½ yrs.

Dose - 25 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy except for interdigital cysts on three feet.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	.5	10,100	5,950,000	14.5		Poor slide			
1.5 hr.	.125								
2.5 hr.	<.03								
3.5 hr.	<.03								
4.5 hr.	<.03	9,200	6,200,000	14.9	4		59	26	11

Table XXXIV Determinations on Dog No. 603 11/20/46

Route - Subcutaneous Male 38 lbs. $2\frac{1}{2}$ yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	4.	11,500	6,510,000	15.1	3		73	17	7
1.5 hr.	.5								
2.5 hr.	.25								
3.5 hr.	.06								
4.5 hr.	< .03								
5.5 hr.	< .03								
6.5 hr.	< .03	12,100	6,290,000	14.9	2		70	18	10

Table XXXVI Determinations on Dog No. 905 11/20/46

Route - Subcutaneous Female 48 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	4.	12,720	7,010,000	14.4	0		60	23	7
1.5 hr.	1.								
2.5 hr.	.5								
3.5 hr.	.125								
4.5 hr.	< .03								
5.5 hr.	< .03								
6.5 hr.	< .03	13,140	6,890,000	14.2	5		65	18	2

Table XXXVII Determinations on Dog No. 904 11/6/46

Route - Subcutaneous Female 45 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy. Carrying considerable adipose tissue.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	4.	14,000	6,700,000	16.5	3		69	19	9
1.5 hr.	1.								
2.5 hr.	.5								
3.5 hr.	.06								
4.5 hr.	< .06								
5.5 hr.	< .06								
6.5 hr.	< .06								
7.5 hr.	< .06								
8.5 hr.	< .06	13,200	7,200,000	15.5	7		66	16	11

Table XXXVIII — Determinations on Dog No. 504 — 11/6/46

Route - Subcutaneous — Male — 36 lbs. — 9 yrs.

Dose - 1000 u/lb. body wt. — Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	13,600	6,750,000	14.1	5		67	20	8
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.125								
4.5 hr.	.06								
5.5 hr.	< .06								
6.5 hr.	< .06								
7.5 hr.	< .06								
8.5 hr.	< .06	14,000	6,000,000	14.4	3		65	20	12

Table XXXIX Determinations on Dog No. 405 11/5/46

Route - Subcutaneous Male 64 lbs. 4 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	12,350	6,100,000	14.4	4		70	18	8
1.5 hr.	1.								
2.5 hr.	.125								
3.5 hr.	<.06								
4.5 hr.	<.06	13,750	6,000,000	15.2	6		71	14	9

Table XL Determinations on Dog No. 205 11/5/46

Route - Subcutaneous Male 52 lbs. 2 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	2.	13,900	6,250,000	16.	2		64	21	13
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.06								
4.5 hr.	<.06	12,650	7,050,000	15.4	5		68	18	9

Table XLI Determinations on Dog No. 104 9/5/46

Route - Subcutaneous Male 35 lbs. 2 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	18,100	6,800,000	15.4	5		72	17	6
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.06								
4.5 hr.	<.03	17,400	6,400,000	15.2	6		72	14	8

Table XLII Determinations on Dog No. 204 9/5/46

Route - Subcutaneous Male 55 lbs. 2 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	13,450	6,200,000	15.6	6		63	19	12
1.5 hr.	.5								
2.5 hr.	.25								
3.5 hr.	.06								
4.5 hr.	<.03	12,400	6,500,000	15.1	4	1	65	20	10

Table XLIII Determinations on Dog No. 302 7/17/46

Route - Subcutaneous Male 33 lbs. 1 yr.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	10,400	6,350,000	15.2	5		64	21	10
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.06								
4.5 hr.	< .06	11,350	6,450,000	16.	3		66	18	13

Table XLIV Determinations on Dog No. 404 7/17/46

Route - Subcutaneous Male 57 lbs. 4 yrs.

Dose - 500 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	12,650	7,000,000	16.2	1		69	25	5
1.5 hr.	1.								
2.5 hr.	.125								
3.5 hr.	<.06								
4.5 hr.	<.06	13,400	6,010,000	15.8	4		68	19	9

Table XLV Determinations on Dog No. 903 8/10/46

Route - Subcutaneous Female 35 lbs. 3 yrs.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy. The dog was beginning to gain weight but was still thin.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	5,500	5,050,000	13.2	4		63	21	12
1.5 hr.	.125								
2.5 hr.	.06								
3.5 hr.	<.06								
4.5 hr.	<.06	6,250	5,650,000	13.4	5		68	18	9

Table XLVI Determinations on Dog No. 003 8/10/46

Route - subcutaneous Female 41 lbs. 10 mo.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100cc.	E.	Differential B.	N.	L.	M.
.5 hr.	.5	13,100	7,020,000	15.2	6		65	18	11
1.5 hr.	.125								
2.5 hr.	<.06								
3.5 hr.	<.06								
4.5 hr.	<.06	12,900	6,500,000	14.9	3		67	20	10

Table XLVII Determinations on Dog No. 303 11/5/46

Route - Subcutaneous Male 32 lbs. 1 yr.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	11,150	6,800,000	14.1	4		72	15	9
1.5 hr	.125								
2.5 hr.	< .06								
3.5 hr.	< .06								
4.5 hr.	< .06	11,850	6,710,000	14.5	2		68	18	12

Table XLVIII Determinations on Dog No. 105 11/5/46

Route - Subcutaneous Male 38 lbs. 2 yrs.

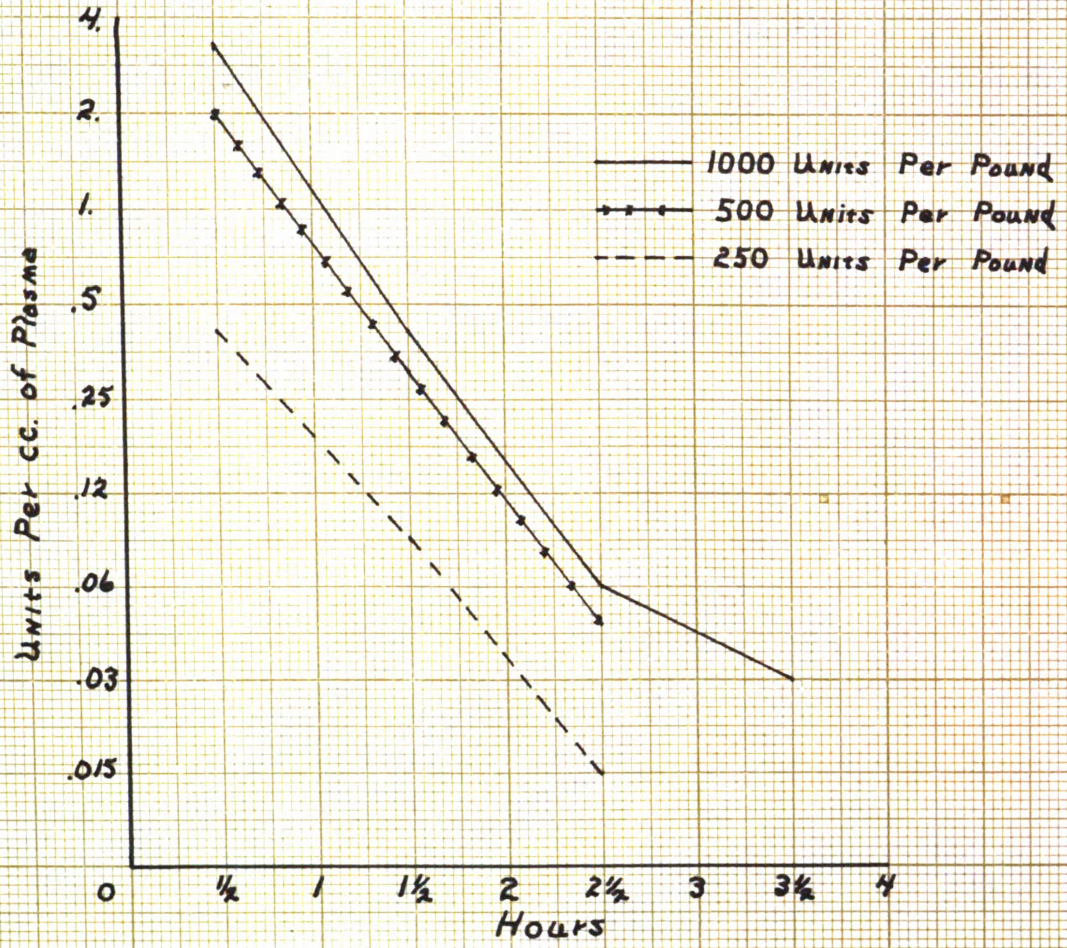
Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

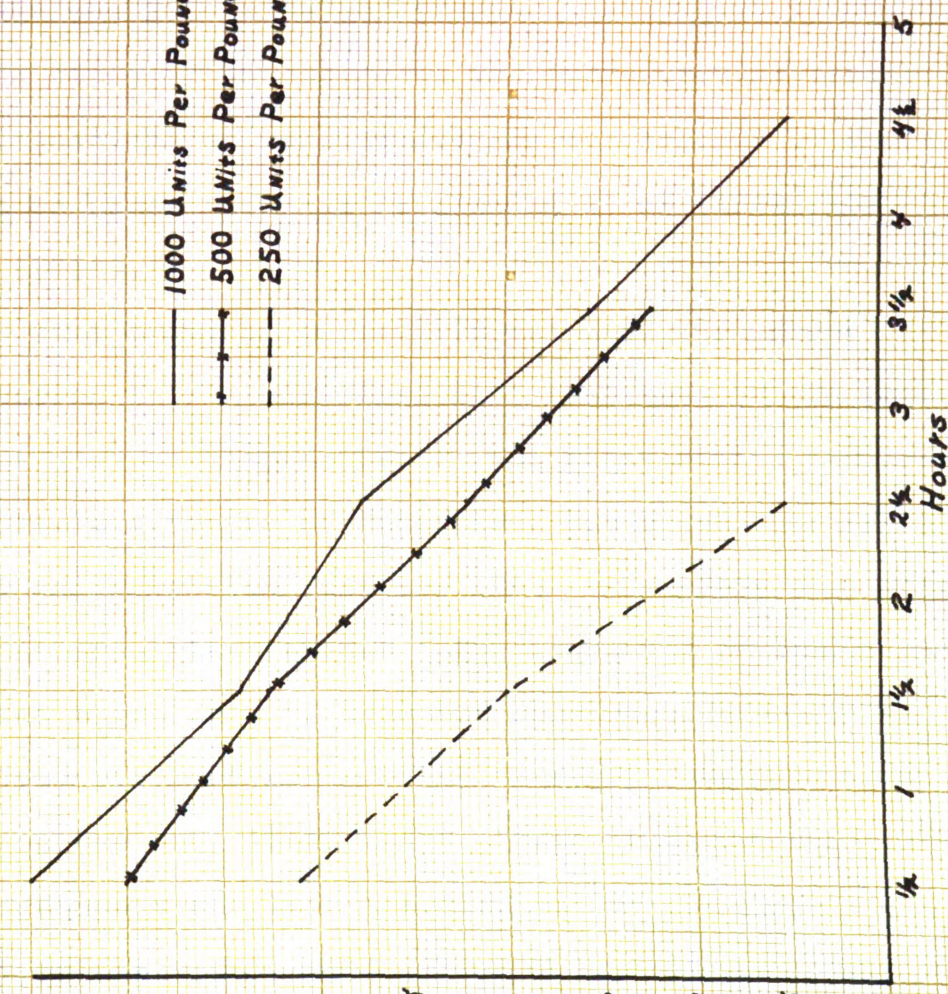
Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	14,300	6,600,000	16.2	2		64	25	9
1.5 hr.	.125								
2.5 hr.	<.06								
3.5 hr.	<.06								
4.5 hr.	<.06	15,050	7,120,000	16.9	6		67	19	8

GRAPH II. AVERAGE PENICILLIN BLOOD PLASMA LEVEL CURVES OBTAINED WHEN SINGLE INJECTIONS OF PENICILLIN IN 5% DEXTROSE AND PHYSIOLOGICAL SALINE WERE ADMINISTERED INTRAMUSCULARLY.



GRAPH III. AVERAGE PENICILLIN BLOOD PLASMA LEVEL CURVES OBTAINED FROM SINGLE INJECTIONS OF PENICILLIN IN 5% DEXTROSE AND PHYSIOLOGICAL SALINE ADMINISTERED SUBCUTANEOUSLY.



decline in penicillin blood plasma level was gradual, dropping below a measurable level at 4.5 to 5.5 hours when the dosage was 1000 units per pound, 3.5 to 4.5 hours when the dosage was 500 units per pound, and 2.5 to 3.5 hours when the dosage was 250 units per pound.

This study of subcutaneous vs. intramuscular penicillin injections at various dosages corresponds to those reported on human patients by Fleming et al (1944) and Hoffman (1946).

The same brand of penicillin was used throughout the course of this study, also different lots of the product were used as they arrived. The first study, comparing the various routes of injection, was begun February 21, 1946 and completed June 13, 1946. The study comparing various dosages given either intramuscularly or subcutaneously was begun July 6, 1946 and completed November 26, 1946. Comparison of the results obtained from these two studies on the penicillin blood plasma concentrations for the subcutaneous and intramuscular routes revealed two distinct points. One point was that much higher penicillin blood plasma levels were obtained from penicillin injections studied after July 6, 1946, the other point was that the measurable levels persisted for a longer period of time.

These observations fell in line with a joint statement reported by the Committee on Medical Research, The United States Health Service, and The Food and Drug Admin-

istration (1946) on the changing character of penicillin. They brought out the fact that commercial penicillin may vary from time to time in the relative content of the various penicillins and that in recent months some commercial penicillins have contained a substantial proportion of penicillin K. Eagle and Musselman (1946) in experimenting with rabbits found that penicillin K disappears from the blood far more rapidly than do other penicillins, thus resulting in a lower initial penicillin blood plasma level and also a shorter measurable level. The joint statement on the changing character of penicillin also pointed out that factors responsible for the apparent decrease in efficacy of commercial penicillin are under intensive study and that practical steps to meet the difficulty are in progress by industrial producers.

The two vehicles for delaying absorption of penicillin which were receiving most publicity at the time of this study were water-in-oil emulsions and oil and wax suspensions. Since very few data have been published on the penicillin levels in dogs following the injection of penicillin in these two vehicles it was decided to compare their delaying effect with that of 5 per cent dextrose in physiological saline as a vehicle.

Two emulsion vehicles were used; one was hydrogenated vegetable oil (Spry*); the other was a product contain-

*Manufactured by Lever Brothers Co., Cambridge, Mass.

Table XLVI Determinations on Dog No. 003 8/10/46

Route - subcutaneous Female 41 lbs. 10 mo.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	13,100	7,020,000	15.2	6		65	18	11
1.5 hr.	.125								
2.5 hr.	<.06								
3.5 hr.	<.06								
4.5 hr.	<.06	12,900	6,500,000	14.9	3		67	20	10

Table XLVII Determinations on Dog No. 303 11/5/46

Route - Subcutaneous Male 32 lbs. 1 yr.

Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	1.	11,150	6,800,000	14.1	4		72	15	9
1.5 hr	.125								
2.5 hr.	<.06								
3.5 hr.	<.06								
4.5 hr.	<.06	11,850	6,710,000	14.5	2		68	18	12

Table XLVIII Determinations on Dog No. 105 11/5/46

Route - Subcutaneous Male 38 lbs. 2 yrs.

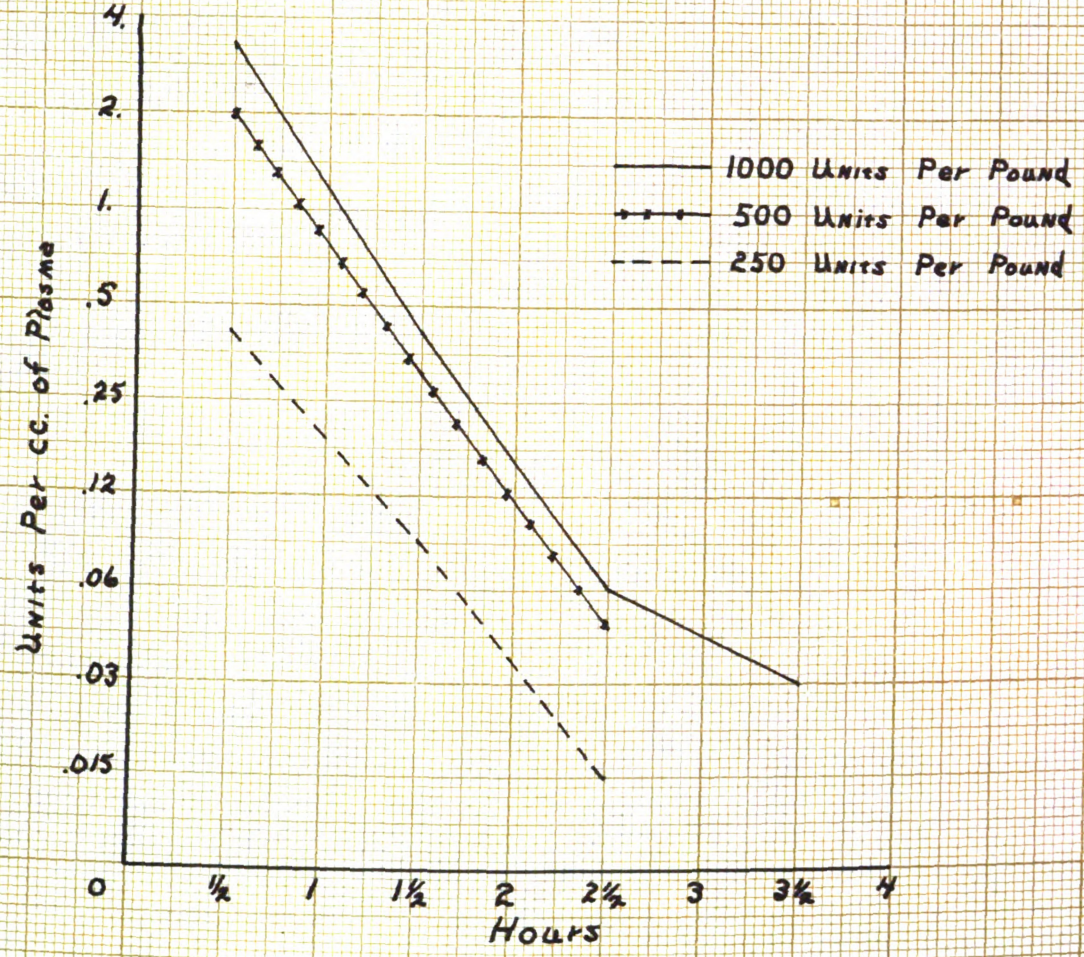
Dose - 250 u/lb. body wt. Vehicle - 5% dextrose in phys. saline.

History - Apparently healthy.

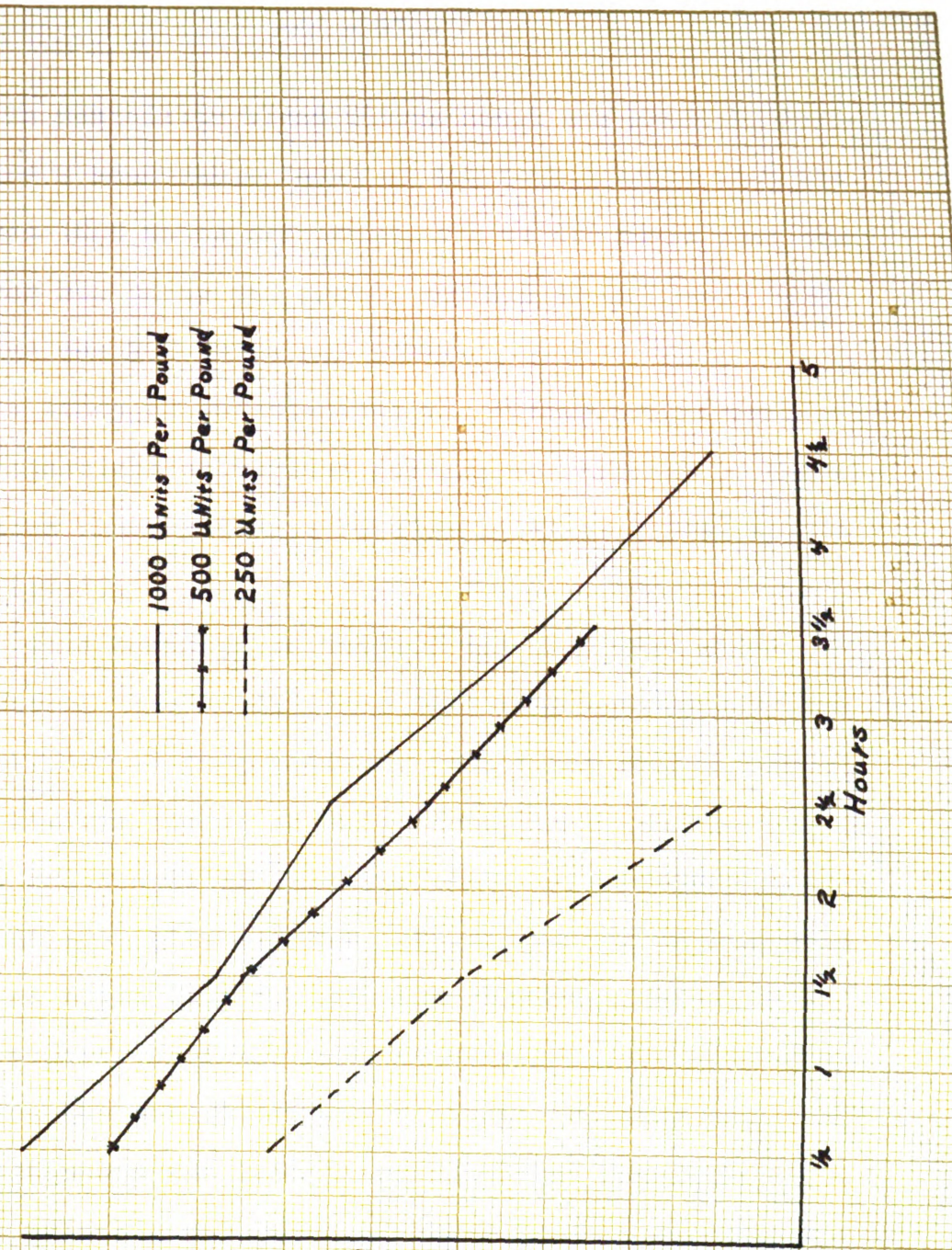
Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	.5	14,300	6,600,000	16.2	2		64	25	9
1.5 hr.	.125								
2.5 hr.	<.06								
3.5 hr.	<.06								
4.5 hr.	<.06	15,050	7,120,000	16.9	6		67	19	8

GRAPH II. AVERAGE PENICILLIN BLOOD PLASMA LEVEL CURVES OBTAINED WHEN SINGLE INJECTIONS OF PENICILLIN IN 5% DEXTROSE AND PHYSIOLOGICAL SALINE WERE ADMINISTERED INTRAMUSCULARLY.



GRAPH III. AVERAGE PENICILLIN BLOOD PLASMA LEVEL CURVES OBTAINED FROM SINGLE INJECTIONS OF PENICILLIN IN 6% DEXTROSE AND PHYSIOLOGICAL SALINE ADMINISTERED SUBCUTANEOUSLY.



decline in penicillin blood plasma level was gradual, dropping below a measurable level at 4.5 to 5.5 hours when the dosage was 1000 units per pound, 3.5 to 4.5 hours when the dosage was 500 units per pound, and 2.5 to 3.5 hours when the dosage was 250 units per pound.

This study of subcutaneous vs. intramuscular penicillin injections at various dosages corresponds to those reported on human patients by Fleming et al (1944) and Hoffman (1946).

The same brand of penicillin was used throughout the course of this study, also different lots of the product were used as they arrived. The first study, comparing the various routes of injection, was begun February 21, 1946 and completed June 13, 1946. The study comparing various dosages given either intramuscularly or subcutaneously was begun July 6, 1946 and completed November 26, 1946. Comparison of the results obtained from these two studies on the penicillin blood plasma concentrations for the subcutaneous and intramuscular routes revealed two distinct points. One point was that much higher penicillin blood plasma levels were obtained from penicillin injections studied after July 6, 1946, the other point was that the measurable levels persisted for a longer period of time.

These observations fell in line with a joint statement reported by the Committee on Medical Research, The United States Health Service, and The Food and Drug Admin-

istration (1946) on the changing character of penicillin. They brought out the fact that commercial penicillin may vary from time to time in the relative content of the various penicillins and that in recent months some commercial penicillins have contained a substantial proportion of penicillin K. Eagle and Musselman (1946) in experimenting with rabbits found that penicillin K disappears from the blood far more rapidly than do other penicillins, thus resulting in a lower initial penicillin blood plasma level and also a shorter measurable level. The joint statement on the changing character of penicillin also pointed out that factors responsible for the apparent decrease in efficacy of commercial penicillin are under intensive study and that practical steps to meet the difficulty are in progress by industrial producers.

The two vehicles for delaying absorption of penicillin which were receiving most publicity at the time of this study were water-in-oil emulsions and oil and wax suspensions. Since very few data have been published on the penicillin levels in dogs following the injection of penicillin in these two vehicles it was decided to compare their delaying effect with that of 5 per cent dextrose in physiological saline as a vehicle.

Two emulsion vehicles were used; one was hydrogenated vegetable oil (Spry*); the other was a product contain-

*Manufactured by Lever Brothers Co., Cambridge, Mass.

ing wax and lanolin in peanut oil*. The emulsions were prepared as reported by Freund and Thompson (1945). The oil and wax suspension used was prepared according to Romansky's Formula containing potassium penicillin.

Single injections of 1,000 units of penicillin per pound of body weight were used as the trial dosage. A limited number of trials were also made using 2,000 units per pound of body weight to see if a more favorable level was sustained at a higher dosage. The subcutaneous route of injection was used as it was the easiest route of administration, least objected to by any of the dogs, and since it was found to be equally effective (when compared with the other routes of administration using 5 per cent dextrose in physiological saline as a vehicle - Tables III to XXII inclusive) in sustaining penicillin blood plasma levels.

The following dogs were used in studying the delaying action of a product containing wax and lanolin in peanut oil as an emulsion vehicle:

1000 units per lb. body weight - L05, 605, 408, 007, B06 and 304.

2000 units per lb. body weight - 406 and 005.

Tables XLIX to LVI inclusive.

The following dogs were used in studying the delay-

*Exact composition unknown.

ing action of Romansky's Formula containing potassium penicillin the following dogs were used:

1000 units per lb. body weight - 805, 206, 407, 006, 306, 208, 108 and 409.

Tables LXV to LXXII inclusive.

Graph IV shows the average penicillin blood plasma level curves for 1000 and 2000 units per pound of body weight when administered in an emulsion using hydrogenated vegetable oil as the vehicle. Graph V shows the same using a product containing wax and lanolin in peanut oil as the vehicle. The average penicillin blood plasma level curve obtained when a single injection of 1000 units per pound of body weight in Romansky's Formula is shown in Graph VI. Graph VI also shows plasma penicillin concentrations after single subcuataneous injections of 1000 units per pound of body weight in the 4 vehicles studied.

Table XLIX Determinations on Dog No. L05 12/10/46

Route - Subcutaneous Female 48 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	14,000	7,060,000	15.2	4		69	19	8
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								

Table L Determinations on Dog No. 605 12/10/46

Route - Subcutaneous Male 38 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	12,050	6,750,000	16.7	4		68	18	10
1.5 hr.	1.								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	<.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	13,300	6,410,000	16.5	7		71	17	5

Table LI Determinations on Dog No. 408 11/27/46

Route - Subcutaneous Male 62 lbs. 4 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	B.	N.	L.	M.
.5 hr.	4.	12,900	5,570,000	15.8	4		69	18	9
1.5 hr.	1.								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	< .03								
6.5 hr.	< .03	13,200	6,200,000	16.0	4		67	20	9

Table LII Determinations on Dog No. 007 11/27/46

Route - Subcutaneous Female 42 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	2.	12,900	5,520,000	14.6	3		68	20	9
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03	13,300	6,110,000	15.2		Poor slide			

Table LIII Determinations on Dog No. B06 11/15/46

Route - Subcutaneous Female 45 lbs. 4 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanoline and peanut oil emulsion.

History - Apparently healthy. Nursing 2 puppies (one month of age).

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	11,800	5,780,000	13.4	5		65	20	10
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.03								
4.5 hr.	< .03								
5.5 hr.	< .03								
6.5 hr.	< .03								
7.5 hr.	< .03								
8.5 hr.	< .03	12,150	6,250,000	13.7	3		67	22	8

Table LIV Determinations on Dog No. 304 11/15/46

Route - Subcutaneous Male 32 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	11,400	6,950,000	14.2	5		62	22	11
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	<.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03	12,950	6,850,000	14.1	4		67	19	10

Table LV Determinations on Dog No. 406 11/13/46

Route - Subcutaneous Male 60 lbs. 4 yrs.

Dose - 2000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
1 hr.	4.	13,000	6,250,000	15.4	4		66	18	12
2 hr.	2.								
3 hr.	.25								
4 hr.	.125								
5 hr.	.06								
6 hr.	.03								
7 hr.	<.03								
8 hr.	<.03								
9 hr.	<.03								
10 hr.	<.03								
11 hr.	<.03								
12 hr.	<.03	13,900	6,900,000	15.0	7		68	14	11

Table LVI Determinations on Dog No. 005 11/13/46

Route - Subcutaneous Female 44 lbs. 1 yr.

Dose - 2000 u/lb. body wt. Vehicle - Wax, lanolin and peanut oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
1 hr.	16.	11,350	6,250,000	15.1	2		66	20	12
2 hr.	4.								
3 hr.	1.								
4 hr.	.25								
5 hr.	.125								
6 hr.	.06								
7 hr.	.03								
8 hr.	<.03								
9 hr.	<.03								
10 hr.	<.03								
11 hr.	<.03								
12 hr.	<.03	11,300	6,000,000	15.4	6		65	18	11

Table LVII Determinations on Dog No. 306 12/10/46

Route - Subcutaneous Male 33 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	13,650	6,600,000	16.5	3		70	21	6
1.5 hr.	.5								
2.5 hr.	.03								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	12,100	6,990,000	16.5	8		63	21	8

Table LVIII Determinations on Dog No. 108 12/10/46

Route - Subcutaneous Male 37 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	17,950	7,550,000	17.5	5		62	23	8
1.5 hr.	.5								
2.5 hr.	.06								
3.5 hr.	.03								
4.5 hr.	<.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	18,700	7,310,000	16.9	2		68	21	9

Table LIX Determinations on Dog No. 207 11/27/46

Route - Subcutaneous Male 53 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	12,350	5,740,000	15.2	2		70	18	10
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03	14,100	6,450,000	15.4	4		65	19	12

Table LX Determinations on Dog No. 806 11/27/46

Route - Subcutaneous Male 54 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	2.	14,050	5,520,000	15.0	5	1	62	23	9
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03	12,950	6,010,000	14.6	2		64	23	11

Table LXI Determinations on Dog No. 106 11/15/46

Route - Subcutaneous Male 38 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	13,150	6,550,000	16.2	8	1	72	15	4
1.5 hr.	1.								
2.5 hr.	.125								
3.5 hr.	.03								
4.5 hr.	< .03								
5.5 hr.	< .03								
6.5 hr.	< .03								
7.5 hr.	< .03								
8.5 hr.	< .03	12,550	6,950,000	16.5	3		72	19	6

Table LXII Determinations on Dog No. L03 11/15/46

Route - Subcutaneous Female 48 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	4.	15,750	6,400,000	14.2	5	2	68	17	8
1.5 hr.	1.								
2.5 hr.	.5								
3.5 hr.	.125								
4.5 hr.	.03								
5.5 hr.	<.03								
6.5 hr.	<.03								
7.5 hr.	<.03								
8.5 hr.	<.03	15,450	6,900,000	14.1	3		68	21	8

Table LXIII Determinations on Dog No. 206 11/13/46

Route - Subcutaneous Male 52 lbs. 2 yrs.

Dose - 2000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
1 hr.	4.	13,750	6,070,000	15.4	3		66	20	1 1
2 hr.	2.								
3 hr.	.5								
4 hr.	.25								
5 hr.	.06								
6 hr.	.06								
7 hr.	.03								
8 hr.	<.03								
9 hr.	<.03								
10 hr.	<.03								
11 hr.	<.03								
12 hr.	<.03	13,700	5,800,000	15.0	7		60	21	12

Table LXIV Determinations on Dog No. 804 11/15/46

Route - Subcutaneous Male 53 lbs. 1 yr.

Dose - 2000 u/lb. body wt. Vehicle - Hydrogenated vegetable oil emulsion.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
1 hr.	8.	14,5000	6,290,000	14.7	4		62	21	13
2 hr.	2.								
3 hr.	.25								
4 hr.	.125								
5 hr.	.03								
6 hr.	.03								
7 hr.	.03								
8 hr.	<.03								
9 hr.	<.03								
10 hr.	<.03								
11 hr.	<.03								
12 hr.	<.03	13,100	5,710,000	14.4	3		67	22	8

Table LXV Determinations on Dog No. 805 11/21/46

Route - Subcutaneous Male 50 lbs. 1 yr.

Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	4.	12,250	5,480,000	14.9	8		61	18	13
1.5 hr.	.5								
2.5 hr.	.125								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	14,350	5,920,000	14.0	3		63	20	14

Table LXVI — Determinations on Dog No. 206 — 11/21/46

Route - Subcutaneous — Male — 51 lbs. — 2 yrs.

Dose - 1000 u/lb. body wt. — Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	12,350	5,760,000	15.0	6		59	25	10
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.06								
4.5 hr.	.03								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	13,800	5,300,000	14.7	4		61	28	7

Table LVII Determinations on Dog No. 407 11/21/46

Route - Subcutaneous Male 63 lbs. 4 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
.5 hr.	2.	12,600	6,510,000	15.6		Poor slide			
1.5 hr.	2.								
2.5 hr.	.25								
3.5 hr.	.125								
4.5 hr.	.06								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	13,300	6,050,000	15.2	7		64	19	10

Table LXVIII — Determinations on Dog No. 006 — 11/21/46

Route - Subcutaneous — Female — 43 lbs. — 1 yr.

Dose - 1000 u/lb. body wt. — Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	10,350	5,940,000	14.9	6		64	20	10
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.03								
4.5 hr.	.03								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	11,000	5,290,000	14.6	4		64	22	10

Table LXIX Determinations on Dog No. 306 1/7/47

Route - Subcutaneous Male 34 lbs. 2 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	E.	Differential B.	N.	L.	M.
15 hr.	2.	13,300	6,900,000	16.0	2		71	20	7
1.5 hr.	2.								
2.5 hr.	.5								
3.5 hr.	.12								
4.5 hr.	.06								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	.03								
8.5 hr.	.03								
9.5 hr.	<.03								
10.5 hr.	<.03	12,300	6,600,000	16.2	8		73	15	4

Table LXX Determinations on Dog No. 208 1/7/47

Route - Subcutaneous Male 50 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	2.	15,100	5,100,000	15.2	3		68	14	5
1.5 hr.	1.								
2.5 hr.	.25								
3.5 hr.	.12								
4.5 hr.	.03								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	.03								
9.5 hr.	<.03								
10.5 hr.	<.03	16,950	5,930,000	15.2	6	1	76	14	3

Table LXXI Determinations on Dog No. 108 1/7/47

Route - Subcutaneous Male 38 lbs. 3 yrs.

Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	20,150	8,110,000	16.9	1		79	13	7
1.5 hr.	2.								
2.5 hr.	.25								
3.5 hr.	.12								
4.5 hr.	.06								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	.03								
8.5 hr.	<.03								
9.5 hr.	.03								
10.5 hr.	<.03	22,5000	7,620,000	16.5	4	1	74	18	3

Table LXXII Determinations on Dog No. 409 1/7/47

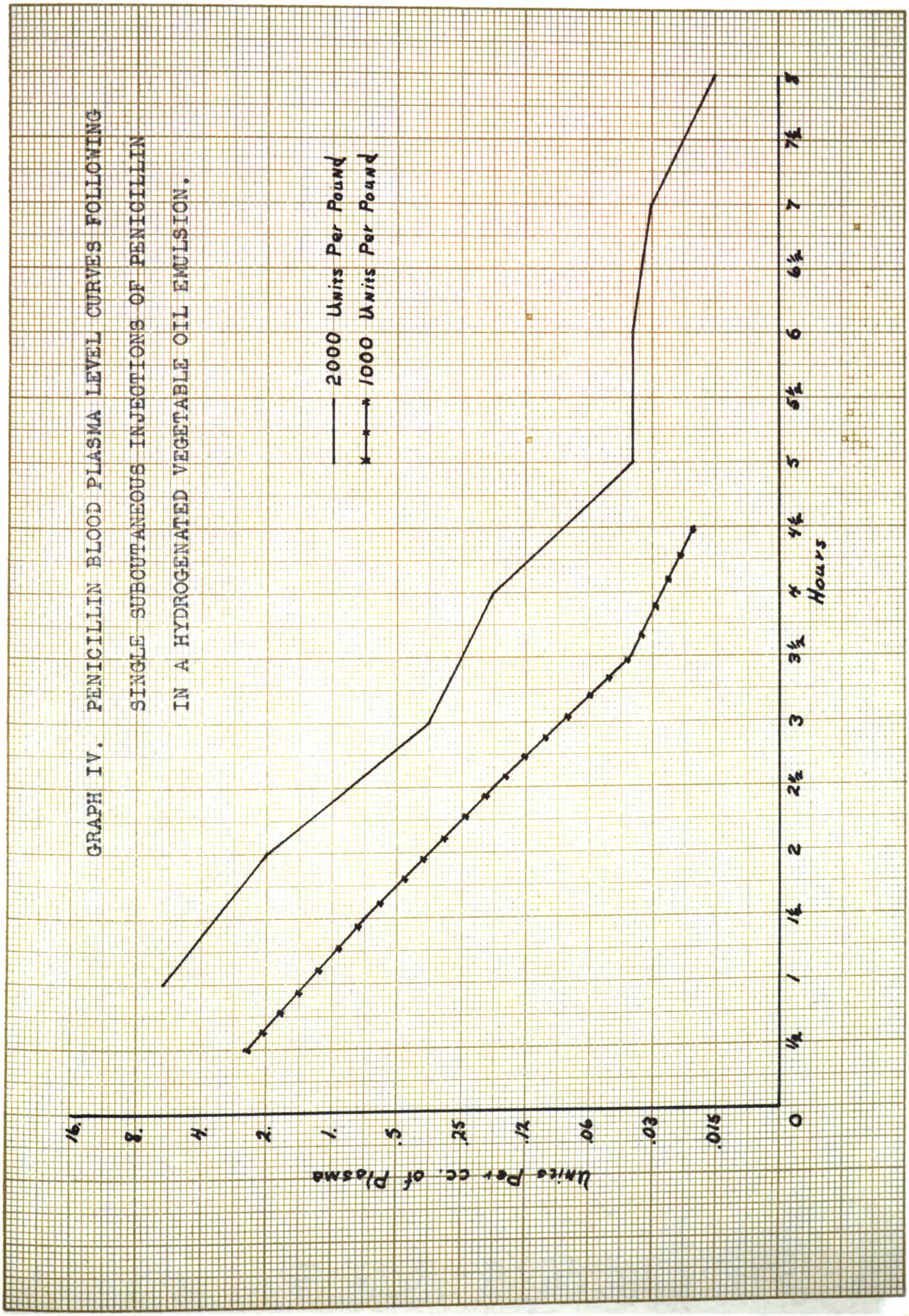
Route - Subcutaneous Male 64 lbs. 5 yrs.

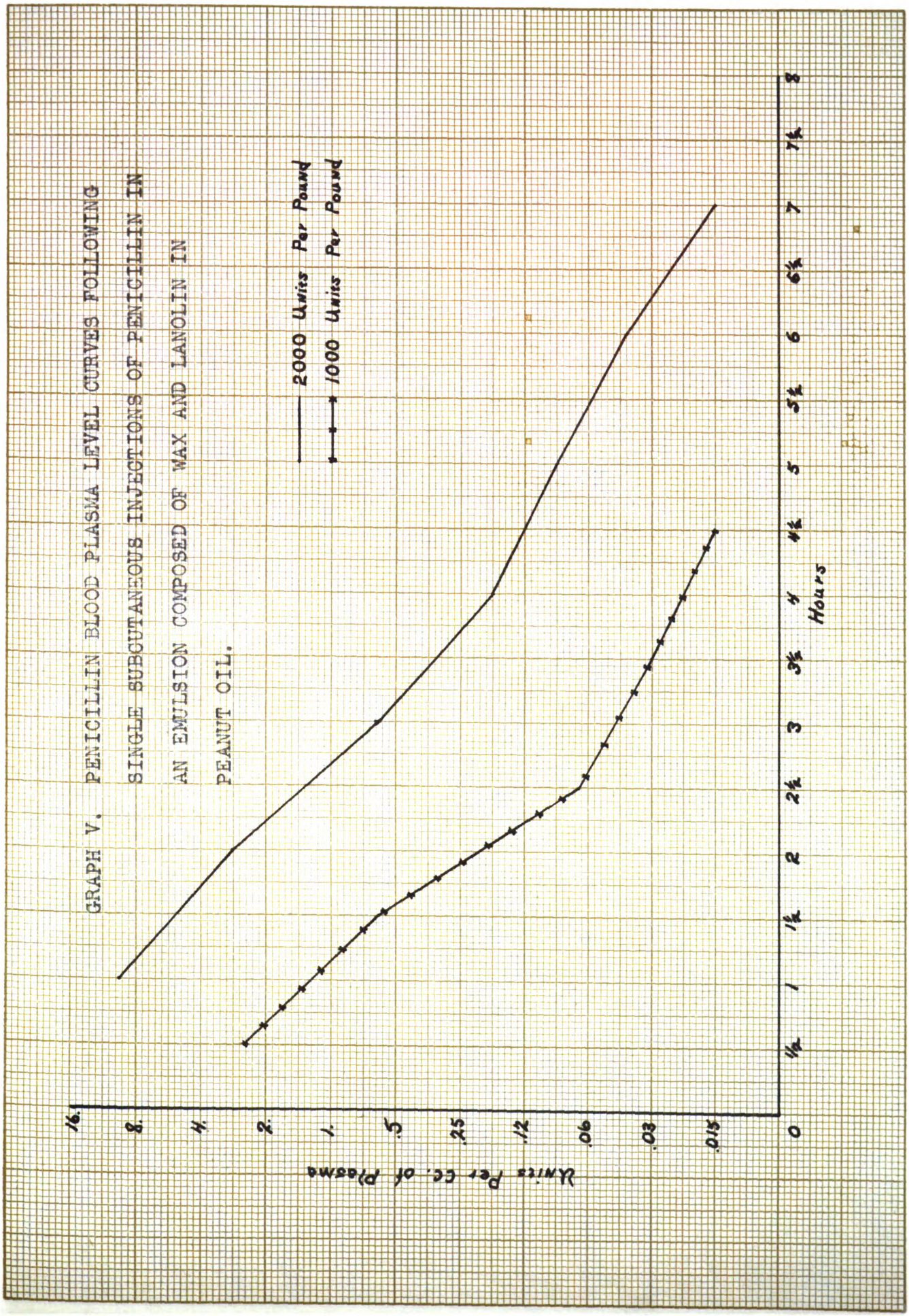
Dose - 1000 u/lb. body wt. Vehicle - Romansky's Formula.

History - Apparently healthy.

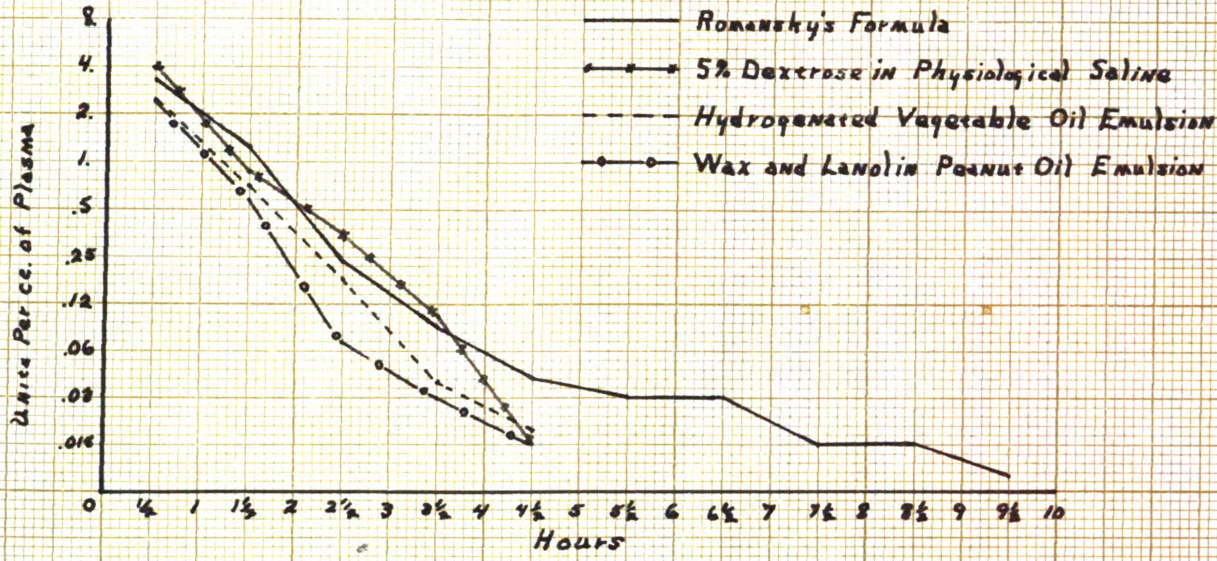
Blood Studies

Time Interval	Penicillin U/cc. plasma	W.B.C.	R.B.C.	Hb. g/100 cc.	Differential E.	B.	N.	L.	M.
.5 hr.	4.	12,250	6,240,000	15.6	7		68	16	9
1.5 hr.	2.								
2.5 hr.	.5								
3.5 hr.	.12								
4.5 hr.	.03								
5.5 hr.	.03								
6.5 hr.	.03								
7.5 hr.	<.03								
8.5 hr.	<.03								
9.5 hr.	<.03								
10.5 hr.	<.03	14,300	5,620,000	15.4	7		69	19	5


GRAPH IV. PENICILLIN BLOOD PLASMA LEVEL CURVES FOLLOWING
SINGLE SUBCUTANEOUS INJECTIONS OF PENICILLIN
IN A HYDROGENATED VEGETABLE OIL EMULSION.
2000 Units Per Pound
1000 Units Per Pound
Units Per cc. of Plasma
16.
8.
4.
2.
1.
.5
.25
.12
.06
.03
.015
0
½
1
1½
2
2½
3
3½
4
4½
5
5½
6
6½
7
7½
8
Hours


GRAPH V. PENICILLIN BLOOD PLASMA LEVEL CURVES FOLLOWING
SINGLE SUBCUTANEOUS INJECTIONS OF PENICILLIN IN
AN EMULSION COMPOSED OF WAX AND LANOLIN IN
PEANUT OIL.
2000 Units Per Pound
1000 Units Per Pound
Units Per cc. of Plasma
16.
8.
4.
2.
1.
.5
.25
.12
.06
.03
.015
0
½
1
1½
2
2½
3
3½
4
4½
5
5½
6
6½
7
7½
8
Hours

GRAPH VI. PENICILLIN BLOOD PLASMA LEVEL CURVES FOLLOWING SINGLE INJECTIONS OF 1000 UNITS PER POUND OF BODY WEIGHT IN VARIOUS VEHICLES ADMINISTERED BY THE SUBCUTANEOUS ROUTE OF INJECTION.



The penicillin blood plasma levels obtained from a single injection of 1000 units per pound of body weight of penicillin in either hydrogenated vegetable oil emulsion or a wax, lanolin and oil emulsion compared very closely. Both resulted in a plasma concentration with a range of 2 to 4 units per cc. of blood at 30 minutes, then declined gradually, dropping below a measurable level at 4.5 to 5.5 hours. When a dosage of 2000 units per pound was used the penicillin concentrations obtained from using the two emulsion vehicles also compared favorably, however, only two trials were run on each vehicle at this dosage.

In the eight trials on Romansky's Formula given subcutaneously at 1000 units per pound the penicillin plasma concentration ranged between 2 and 4 units at 30 minutes. From this point on the decline was gradual, values dropping below a measurable level at 7.5 hours for 5 of the 8 dogs. Of the three remaining one dropped below the measurable level at 9.5 hours, another dropped below at 7.5 hours, showed a level of 0.03 units at 8.5 hours then dropped below at 9.5 hours, the third dropped below at 8.5 hours, showed a level of 0.03 units at 9.5 hours, then dropped below at 10.5 hours. This irregularity of absorption was noted by Kirby et al (1945) in human patients who maintained assayable levels for a longer period of time.

In delaying the absorption of penicillin the oil and wax suspension of penicillin was found to be superior to the water-in-oil emulsion. The suspension given at the

rate of 1000 units per pound maintained a level of 0.03 units or better for 6.5 hours while the emulsion was found to maintain this level for only 3.5 hours. When 2000 units per pound was administered in either of the emulsions used a level of 0.03 units per cc. was sustained 5.5 to 7.5 hours. When compared to 5 per cent dextrose in physiological saline as a vehicle, neither of the emulsions was found to possess any advantage over the former vehicle in delaying absorption of penicillin.

During the course of these experiments one opportunity (Dog No. B05 - Table LXXIII) was presented to study the transmission of penicillin to the amniotic fluid and fetal blood following an injection of penicillin into the pregnant bitch. Two-thousand units per pound of body weight was given in water-in-oil emulsion and administered by the subcutaneous route. Hourly blood samples were drawn from the bitch. Four hours after the initial injection of penicillin, the bitch was given ether anesthesia and the young were removed by caesarian section. Samples of the amniotic fluid and blood were taken from each of the first five puppies as they were removed from the uterus. The last two puppies were kept and not sacrificed for blood samples, however, samples were taken of the amniotic fluid.

No evidence of toxicity was observed either in the bitch or in the two puppies that were saved as all three

Table LXXIII — Determinations on Dog No. B05 — 10/15/46

Route - Subcutaneous — Female — 50 lbs. — 4 yrs.

Dose - 200,000 u/lb. body wt. — Vehicle - Wax, lanolin and peanut oil emulsion.

History - The bitch was injected at 12 noon. Hourly blood samples were drawn. Caesarian section was performed at 4 P.M. Samples of amniotic fluid were taken from each fetal amnion. Blood samples were taken from 5 of the puppies.

Blood Studies on Bitch

Time Interval	Penicillin U/cc. plasma
1 hr.	2.
2 hr.	1.
3 hr.	1.
4 hr.	.5
5 hr.	.25
6 hr.	.125
7 hr.	.06
8 hr.	<.06
9 hr.	<.06
10 hr.	<.06

Blood Studies on Puppies

Penicillin U/cc. Amniotic fluid		Penicillin U/cc. Puppies plasma	
A-1	<.06	B-1	.06
A-2	.06	B-2	.06
A-3	.06	B-3	<.06
A-4	.06	B-4	<.06
A-5	.06	B-5	.06
A-6	.06		
A-7	.06		

made an uneventful recovery and the two puppies grew normally.

The mother's penicillin blood plasma concentration at one hour following injection of penicillin was 2 units per cc. At four hours, when the caesarian section operation was begun, her level was 0.5 units, dropping below a measurable level at 8 hours. The amniotic fluid from 6 out of the 7 amnions had a penicillin concentration of 0.06 units. Blood plasma from 3 of the 5 puppies checked had a penicillin blood plasma concentration of 0.06 units.

Seventy-one penicillin trials were run during the course of this experiment. In 70 of these, blood samples were taken at the beginning and end of each individual trial and checked for red and white cell counts and hemoglobin content. Differential leukocyte counts were made on 67 of the dogs, in three cases the slides were too poorly stained for accurate counting.

The greatest difference between the initial and final count in any one trial was 890,000 red blood cells per cu. mm., 2,350 white blood cells per cu. mm. and 1.0 gram of hemoglobin. On the differential counts, in any one given trial, the largest difference between initial and final counts on eosinophiles was 6 cells, neutrophiles 12 cells, lymphocytes 7 cells and monocytes 8 cells.

Todd and Sanford (1943) state that "Since twice the Standard Deviation is the usually accepted limits of significance, the error of a single estimate of the erythrocyte

count was given by them as ± 16 per cent". The difference between the initial and final erythrocyte counts in each case was found to be less than the "normal" error allowable.

Each trial was further tabulated to find out if there was a trend towards lowering or raising of the cellular constituents or hemoglobin content following a single injection of penicillin. Results of this tabulation are shown in Table LXXIV.

Table LXXIV. BLOOD PICTURE FOLLOWING A SINGLE INJECTION OF PENICILLIN COMPARED WITH THE INITIAL BLOOD PICTURE.

Test	No. of Trials	Showing Decrease	Showing Increase	Trials Showing Identical Results
W. B. C.	70	31	36	3
R. B. C.	70	39	29	1
Hemoglobin	70	35	30	5
% Eosinophiles	67	28	35	4
% Basophiles	67	5	5	57
% Neutriphiles	67	35	27	5
% Lymphocytes	67	27	34	6
% Monocytes	67	26	31	10

The results revealed no observable trends as the number of trials showing an increase approximately balanced with those showing a decrease. Since the difference bet

tween the initial and final results in each trial was found to be more or less within the normal error and since there was no trend toward raising or lowering, a single injection of penicillin was thought to have no immediate effect (within 3 to 12 hours) on altering red and white cell counts, differential counts or hemoglobin content in the normal healthy dog. The range of blood constituents in all trials was found to be within the normal variations reported by Coffin (1945), Malkamus (1944) and Boddie (1946).

The subcutaneous route of injection was found to be the one of choice for ease of administration. None of the dogs objected to this route when a 24 gauge needle was used. Slight objection was registered by a few of the dogs when the 17 gauge needle was used for injection of the oil and wax emulsions and suspensions. Objection here was to the insertion of the needle and not to the deposition of the penicillin and vehicle. All dogs objected to the intramuscular and intraperitoneal injections. Intravenous injections were well tolerated.

None of the dogs exhibited any local or systemic reactions following the injection of penicillin, regardless of the vehicle used.

All dogs were sacrificed for autopsy at the termination of the experiment. Examinations of the sites of injection revealed no macroscopic lesions in any of the animals.

SUMMARY AND CONCLUSIONS

Published experimental data obtained by the administration of penicillin in dogs using the various injectable routes, different dosages and vehicles were found to be quite limited. Data on blood cell counts and hemoglobin content following administration of penicillin were also limited.

The vehicles used in this experiment were: 5 per cent dextrose in physiological saline, two water-in-oil emulsions (hydrogenated vegetable oil, wax and lanolin in peanut oil) and Romansky's Formula.

The dogs exhibited the least objection to injections by the subcutaneous and intravenous routes and most objection to those by the intramuscular and intraperitoneal routes when penicillin was administered. Following a single injection of 1000 units per pound of body weight in a vehicle consisting of 5 per cent dextrose in physiologicalsaline the penicillin blood plasma concentration was found to be approximately the same for all four routes of injection at 30 minutes, however,the penicillin level of 0.03 units or better was maintained approximately twice as long when the sucutaneous, intramuscular, and intraperitoneal routs were used as compared to the intravenous route.

Penicillin blood plasma levels 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 were studied. 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.

A change in the character of penicillin was noted, as that used after the middle of June 1946 gave rise to a higher level and maintained a measurable level somewhat longer.

No local or systemic reactions were noted following the injection of the various penicillin preparations used in this experiment. Studies on the blood picture indicate that single injections of penicillin in dosages from 250 to 2000 units per pound of body weight have no immediate

effect on altering the red and white cell counts, differential count or hemoglobin content in the normal healthy dog. No macroscopic lesions were found at the sites of injection on autopsy of the dogs at the termination of the experiment.

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