COMPARISON OF THE EFFICACY OF THERAPEUTIC AGENTS IN THE TREATMENT OF EXPERIMENTALLY INDUCED DIFFUSE PERITONITIS OF INTESTINAL ORIGIN*

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AN OPERATION DESIGNED TO PRODUCE a fulminating rapidly fatal peritonitis of intestinal origin was performed on 111 dogs. It was necessary to exclude 18 animals from the group because of complications that interfered with the experiments such as death from anesthesia, air embolism during intravenous therapy, internal fecal fistula, appendiceal avulsion, and hemorrhage. The remaining 93 animals were considered satisfactory for the purpose of evaluating therapeutic agents for the treatment of experimental peritonitis of appendiceal origin.

METHOD

A method that had previously been developed in this laboratory was used. Laparotomy was performed under sodium pentobarbital anesthesia; the blood supply to the appendix was divided and ligated; feces was expressed into the appendix, filling its lumen completely; the base of the appendix was ligated with flat cotton tape ¼ inch in width; the appendix was crushed by clamping it repeatedly with a large Kocher type hemostat; the spleen and omentum were removed; the animal was given 60 cc. of castor oil by gavage. A small soft rubber tube was placed in the peritoneal cavity and brought out through the laparotomy incision. With the use of aseptic precautions peritoneal fluid for bacterial cultures was aspirated through this tube which was then removed. (Preliminary sampling experiments had revealed definite gross evidence of peritonitis and positive bacterial cultures to be uniformly present six hours after operation.)

The tube also served in some experiments as the means for introducing intraperitoneal therapy. In such experiments, the tube was left in place until the intraperitoneal therapy was discontinued. At the time the tube was removed the skin and subcutaneous tissues were sutured to prevent leakage of peritoneal fluid through the wound. In most of the animals the tubes functioned satisfactorily, but occasionally an animal would withdraw the tube before the experiment was completed.

Simultaneous determinations of blood and peritoneal fluid levels of chemotherapeutic agents were made in some experiments in order to determine

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concentrations and blood-peritoneal fluid relationships during the period of treatment of the infection. The dosage and method of administration of the various chemotherapeutic agents used are described separately with each group of experiments. All experimental animals were given parenteral fluids postoperatively to maintain adequate water and electrolyte intake.

Temperature recordings and leucocyte counts were made in the early experiments but were not done later as they usually were found to be elevated above normal, and this fact did not contribute significantly to the experiment. The appearance, illness, toxicity, and the behavior and habitus of the animals were found to be more valuable indications of the progress of the infection.

Autopsies with bacteriologic and pathologic studies were done at once on all animals that died. Laparotomies with bacteriologic and pathologic studies were performed at weekly intervals on all surviving animals.

CONTROLS

Twenty animals served as controls. All became acutely ill, vomited, grew progressively lethargic and toxic, became comatose and died. The average

TABLE I.—*Experimental Peritonitis*

Untreated Control Series (20 animals) Organisms Cultured	Number of Animals	
Escherichia coli	19	
Proteus vulgaris	2	
Bacterioides	2	
Aerobic sporeforming bacilli	1	
Clostridia	15	
Alpha hemolytic streptococcus	3	16
Beta hemolytic streptococcus	5	
Streptococcus fecalis	8	
Hemolytic *staphylococcus aureus*	1	10
Nonhemolytic *staphylococcus albus*	6	
Hemolytic *staphylococcus albus*	3	

survival period was 39 hours. Autopsies revealed a diffuse acute inflammatory process throughout the peritoneum. The peritoneal cavity contained thin sanguineous exudate estimated at from 100 cc. to 1000 cc. Varying amounts of fibrinous exudate were deposited about the peritoneal cavity. The appendix was covered with yellow-green exudate and there was no evidence of localization or of a "walling off" process about the appendix.

The bacteria cultured from peritoneal fluid in the untreated control animals are listed in Table I. The organisms most often found were Clostridia, Streptococci and *Escherichia coli.*

GROUP I. INTRAVENOUS SODIUM SULFADIAZINE

Five dogs in which appendiceal peritonitis had been produced were treated with intravenous sodium sulfadiazine. Therapy was started six hours post-

operatively. Each animal received 4.0 Gm. of sodium sulfadiazine twice daily with Sodium R Lactate 1/6 M to maintain an alkaline reaction in the urine. There was no apparent beneficial effect from sodium sulfadiazine therapy. The progress of the infection was identical with that in untreated control animals. All died. The average survival period was 44 hours. Table II indicates the types of organisms cultured before therapy and at autopsy.

TABLE II.—*Experimental Peritonitis*
Group I. 5 animals.

Sodium sulfadiazine 4.0 Gm. given twice daily, intravenously

Organisms Cultured	Incidence: 6 Hrs. Postop. (3 Dogs)	Incidence: Necropsy (5 Dogs)
Escherichia coli	1	4
Proteus vulgaris	0	2
Aerobic sporeforming bacillus	1	0
Clostridia	2	5
Alpha hemolytic streptococcus	0	2
Streptococcus fecalis	0	3
Beta hemolytic streptococcus	0	1
Gamma streptococcus	1	0
Nonhemolytic *staphylococcus albus*	0	2
Hemolytic *staphylococcus albus*	1	0

TABLE III.—*Experimental Peritonitis*
Group I.

Blood and peritoneal fluid sulfadiazine levels (milligrams per cent) following the administration of 4.0 Gm. of sodium sulfadiazine intravenously

Hours	Blood	Peritoneal Fluid
0	0	0
2	24.0	24.0
4	23.0	23.0
6	23.0	23.0
8	23.0	23.0

TABLE IV.—*In vitro Sulfadiazine Susceptibility*
Group I.

Organisms Cultured	Milligrams Per Cent
Escherichia coli	8
Proteus vulgaris	8
Alpha hemolytic streptococcus—Not susceptible to	10
Beta hemolytic streptococcus	8
Gamma streptococcus	8
Streptococcus fecalis	Not susceptible
Clostridia	
Hemolytic *staphylococcus albus*	8
Nonhemolytic *staphylococcus albus*	8

The concentrations of sulfadiazine in the blood and peritoneal fluid in this group are shown in Table III.

Table IV indicates the *in vitro* sulfadiazine susceptibility of the individual organisms cultured from these experiments.

GROUP II. INTRAPERITONEAL SULFANILAMIDE COMBINED WITH INTRAVENOUS SULFADIAZINE

Five dogs in which appendiceal peritonitis had been produced were treated with intravenous sulfadiazine therapy exactly as described in the preceding group of experiments. In addition, 5.0 Gm. of sulfanilamide were given intraperitoneally through the rubber tube six hours postoperatively just prior to removal of the tube.

Table V shows the blood sulfanilamide concentration following the intraperitoneal instillation of 5.0 Gm. of sulfanilamide.

TABLE V.—*Experimental Peritonitis*
Group II.

Blood sulfanilamide levels following intraperitoneal instillation of 5.0 Gm. of sulfanilamide

Time	Blood Level (mg. %)
0	0
15 minutes	6.20
1½ hours	14.05
4 hours	15.35
6 hours	13.29
8 hours	15.50
24 hours	6.78

Although the survival period was prolonged in this group (average 80 hours) all animals died. The course of illness and the pathological findings were similar to the untreated controls.

The types of organisms cultured are shown in Table VI. Sulfadiazine mixed culture tests showed most of these organisms to be resistant to sulfadiazine in concentrations up to 12,500 mg. per cent, and sulfanilamide mixed culture tests showed all organisms to be resistant to concentrations of sulfanilamide up to 200 mg. per cent.

TABLE VI.—*Experimental Peritonitis*
Group II. 5 animals.

Sodium sulfadiazine intravenously, 4.0 Gm. twice daily, and sulfanilamide, 5.0 Gm. six hours post-operatively, intraperitoneally

Organisms Cultured	Incidence: 6 Hrs. Postop. (5 Dogs)	Incidence: Necropsy (5 Dogs)
Escherichia coli	1	4
Clostridia	1	3
Streptococcus fecalis	4	5
Hemolytic *staphylococcus aureus*	2	1
Hemolytic *staphylococcus albus*	2	3
Hemolytic *staphylococcus citrous*	1	0
Diphtheroids	2	0
Aerophilic lactobacilli	0	2

At autopsy peritoneal fluid sulfadiazine levels were found to vary from 44.0 to 182.0 mg. per cent.

GROUP III. INTRAPERITONEAL SULFASUXIDINE

Five dogs in which experimental appendiceal peritonitis had been produced were treated with intraperitoneal instillations of sulfasuxidine (1.0 Gm. per kilogram of body weight) once daily beginning six hours postoperatively. All animals died. The course of illness, period of survival (average 40 hours) and necropsy findings were similar in every respect to the untreated control series.

Table VII shows the bacteriology in this group of experiments.

TABLE VII.—*Experimental Peritonitis*
Group III. 5 animals.

Sulfasuxidine 1.0 Gm. per kg. per day intraperitoneally			
	Incidence		
Organisms Cultured	6 Hrs. Postop. (5 dogs)	1 Day Postop. (2 dogs)	Necropsy (5 dogs)
Escherichia coli	2	1	5
Proteus vulgaris	..	1	..
Clostridia	3	1	2
Streptococcus fecalis	4	2	3
Staphylococcus aureus	2	0	0
Staphylococcus albus	2	0	1
Diphtheroids	..	..	1

Undissolved sulfasuxidine varying from small to considerable amounts was found spread throughout the peritoneal cavity at autopsy. Occasionally considerable accumulations of the drug were found to be pocketed in fibrin. The concentration of sulfasuxidine in the peritoneal fluid at autopsy was found to be from 6.0 to 42.0 mg. per cent (measured as sulfathiazol). Table VIII shows increasing sulfasuxidine resistance of the bacterial flora and the concentration of the drug in these experiments.

TABLE VIII.—*Sulfasuxidine Susceptibility Tests*
Group III.

	6 Hours Postoperatively	Necropsy
Escherichia coli	Susceptible 3.12– 6.24 mg.%	Most susceptible at 50.0 mg.%
Proteus vulgaris		Not susceptible at 50.0 mg.%
Clostridia	Susceptible 0.28–50.0 mg.%	Not susceptible at 50.0 mg.%
Streptococcus fecalis	Most unsusceptible to 50.0 mg.%	Some not susceptible and some susceptible to 50.0 mg.%
Staphylococcus aureus	Susceptible 0.25–50.0 mg.%	Not susceptible at 50.0 mg.%
Diphtheroids		Not susceptible at 50.0 mg.%

GROUP IV. STREPTOMYCIN
(INTRAMUSCULARLY BEGINNING SIX HOURS POSTOPERATIVELY)

Ten dogs in which appendiceal peritonitis had been produced were treated with intramuscular injections of streptomycin. Therapy was started six hours postoperatively. Each animal received 2 Gm. of streptomycin daily in divided

doses of .33 Gm. given intramuscularly at four-hour intervals. Two of the ten animals recovered from the acute peritonitis under streptomycin therapy, but a fecal fistula suddenly developed in one of the animals on the sixth postoperative day and it expired the following day. The remaining eight animals died with the same acute signs of peritonitis as the controls, but their average survival period was 92 hours, as compared to an average survival period of 39 hours for the untreated control animals. The only pathogenic organisms that were consistently susceptible to the streptomycin in vitro were *Escherichia coli* and they usually persisted in their growth in vivo and were found at autopsy. The Streptococci and Clostridia were uniformly resistant to streptomycin both in vitro and in vivo.

TABLE IX.—*Experimental Peritonitis*
Group IV. 10 animals.

Streptomycin 2.0 Gm. daily (.33 Gm. intramuscularly every 4 hours). Started 6 hours postoperatively	Incidence	
Organisms Cultured	6 Hrs. Postop. (7 dogs)	Necropsy (9 dogs)
Escherichia coli	6	9
Proteus vulgaris	0	1
Bacterioides	1	0
Aerobic sporeforming bacillus	1	1
Clostridia	5	8
Alpha hemolytic streptococcus	1	1
Beta hemolytic streptococcus	2	4
Gamma streptococcus	2	1
Streptoccus fecalis	1	2
Nonhemolytic *staphylococcus albus*	2	2
Hemolytic *staphylococcus albus*	1	1
Hemolytic *staphylococcus aureus*	1	1

The one streptomycin-treated animal that survived without complications was lost inadvertently during induction of anesthesia prior to exploratory laparotomy on the seventh postoperative day. Gross examination of the peritoneal cavity revealed evidence of subsiding peritonitis. Cultures of the peritoneal exudate taken before the streptomycin therapy was started grew the usual bacterial flora of the colon.

Table IX shows the types of organisms cultured from the animals in this experiment.

Table X gives the streptomycin susceptibility of the bacterial organisms cultured from the peritoneal cavity prior to treatment of this group of animals with streptomycin.

Table XI shows blood and peritoneal fluid streptomycin concentrations following intramuscular administration of streptomycin.

GROUP V. STREPTOMYCIN
(INTRAMUSCULARY BEGINNING IMMEDIATELY POSTOPERATIVELY)

Five dogs in which appendiceal peritonitis had been produced were treated with streptomycin beginning immediately after operation. Each animal was

given 2 Gm. of streptomycin daily in divided doses of .33 Gm. intramuscularly every four hours. The course of infection in these animals was similar to that in the untreated control animals. All died. However, the average survival period of these animals treated with streptomycin was 75 hours as compared with an average survival period of 39 hours for the untreated control animals.

Table XII indicates the bacteria cultured in this group of experiments.

TABLE X.—*Experimental Peritonitis*
Group IV.

Organisms	Streptomycin susceptibility Units per Cubic Centimeter
Escherichia coli	1.78–20.0
Proteus vulgaris	7.1 –80.0
Bacterioides	Not susceptible
Aerobic sporeforming bacillus	3.0
Pseudomonas aeruginosa	Not susceptible
Clostridia	Not susceptible
Alpha hemolytic streptococcus	Not susceptible
Beta hemolytic streptococcus	1.3 –28.5
Gamma streptococcus	3.57
Streptococcus fecalis	Not susceptible
Non hemolytic *staphylococcus albus*	14.6
Hemolytic *staphylococcus albus*	3.0 – 8.0
Hemolytic *staphylococcus aureus*	2.0

TABLE XI.—*Experimental Peritonitis*
Group IV.

Blood and peritoneal fluid streptomycin concentrations (units per cubic centimeter) following the administration of 0.33 Gm. of streptomycin intramuscularly

Hours	Blood	Peritoneal Fluid
0	0	0
1	20	0
2	40	20
3	40	20
4	20	10

TABLE XII.—*Experimental Peritonitis*
Group V. 5 animals.

Streptomycin 2.0 Gm. daily (.33 Gm. intramuscularly every 4 hours) beginning immediately after completion of operation

Organisms Cultured	Incidence: 6 Hrs. Postop. (4 dogs)	Incidence: Necropsy (5 dogs)
Escherichia coli	2	3
Bacterioides	0	1
Clostridia	3	4
Alpha hemolytic streptococcus	0	1
Beta hemolytic streptococcus	1	0
Gamma streptococcus	1	0
Streptococcus fecalis	2	3
Nonhemolytic *staphylococcus albus*	2	0
Nonhemolytic *staphylococcus aureus*	1	0
Diphtheroid bacillus	2	0

GROUP VI. STREPTOMYCIN INTRAPERITONEALLY

Ten dogs in which peritonitis was produced were treated with intraperitoneal instillation of streptomycin. Therapy was started six hours postoperatively.

A. In the first group of five animals, three were given 0.4 Gm. intraperitoneally on two occasions on the day of operation, and 0.4 Gm. daily thereafter. Two animals were given 0.8 Gm. twice daily on the day of operation, and 0.8 Gm. once daily thereafter. All animals died with acute peritonitis. The course of illness and autopsy findings were the same as those in the untreated control animals. However, the average survival period of the animals treated with intraperitoneal streptomycin was 87 hours compared with an average survival period of 39 hours for the untreated controls.

Table XIII shows the bacteriologic findings six hours postoperatively and at necropsy in this group of experiments. Additional cultures taken at 22, 30, 48 and 72 hours postoperatively were similar to those made at necropsies.

TABLE XIII.—*Experimental Peritonitis*
Group VI-A 5 animals.

Streptomycin intraperitoneally 0.4 to 1.6 Gm. daily	Incidence	
Organisms Cultured	6 Hrs. Postop. (4 dogs)	Necropsy (5 dogs)
Escherichia coli	2	4
Clostridia	3	5
Alpha hemolytic streptococcus	2	4
Gamma streptococcus	0	1
Streptococcus fecalis	1	0
Nonhemolytic *staphylococcus albus*	2	1

B. Up to this point in vitro laboratory tests and animal experiments had demonstrated considerable evidence that the pathogenic bacterial flora of experimental peritonitis were resistant to streptomycin in concentrations and doses that ordinarily would be considered safe to use in therapy of human patients. It was decided to attempt to use intraperitoneally doses of streptomycin that would be more likely (based on in vitro streptomycin susceptibility tests) completely to destroy all pathogenic organisms. Four (4.0) Gm. of streptomycin were instilled intraperitoneally in each of five dogs six hours after completion of the operation to produce experimental peritonitis. All dogs died within 10 to 30 minutes after either their first or second instillation of streptomycin. The average survival period was 12 hours. These deaths occurred from respiratory failure due to the toxic effect of streptomycin on the medullary centers. Blood streptomycin concentration was 160 units per cc. 10 minutes after intraperitoneal instillation of 4.0 Gm. of streptomycin.

Bacterial cultures of the peritoneal fluid were taken at necropsy in four of the animals in this group. In three animals the cultures were sterile. Escherichia coli and Clostridia were cultured from the peritoneal fluid

specimen taken at the necropsy of the fourth animal. All animals had gross evidence of acute diffuse peritonitis at autopsy.

Table XIV indicates the bacteriologic findings in this group of experiments.

TABLE XIV.—*Experimental Peritonitis*
Group VI-B 5 animals.

Streptomycin intraperitoneally 4.0 Gm. twice daily beginning 6 hours postoperatively

Organisms Cultured	Incidence	
	6 Hrs. Postop. (4 dogs)	Necropsy (4 dogs)
Escherichia coli	3	1
Clostridia	3	1
Alpha hemolytic streptococcus	2	0
Beta hemolytic streptococcus	1	0
Gamma streptococcus	1	0
Hemolytic *staphylococcus aureus*	1	0

GROUP VII. PENICILLIN

Commercially available penicillin was given intramuscularly in divided doses every four hours to three groups of animals. Penicillin therapy was started six hours following operations for the production of experimental peritonitis of appendiceal origin. Each animal in the first group received 100,000 units of penicillin per day, the second group 200,000 units of penicillin per day, and the third group 500,000 units of penicillin per day. All animals that survived received treatment for six days.

A. One out of three animals in the first group (treated with 100,000 units of penicillin daily for six days) survived and two died with diffuse peritonitis. The course of illness, pathology and bacteriologic cultures in the animals that died were similar to those seen in the untreated control animals. The one animal that survived had gross pathologic evidence of a subsiding peritonitis when examined at exploratory laparotomy on the seventh day. Bacteriologic cultures made on that day grew Gamma streptococci and Clostridia. Apparently the laparotomy reactivated the infection for the animal died five days later with acute diffuse peritonitis (no attempt was made to treat this animal with penicillin following the exploratory laparotomy).

B. In the second group (treated with 200,000 units of penicillin daily for six days) two animals survived and three died with acute diffuse peritonitis. The course of the disease, pathologic and bacteriologic findings in the animals that died were similar to those in the untreated control series. The animals that survived had positive bacterial cultures and gross evidence of subsiding acute peritonitis at exploratory laparotomies performed on the seventh day. One survivor died four days after exploratory laparotomy with fulminating diffuse peritonitis. The other survivor was examined at necropsy one month postoperatively (during this period of one month the animal appeared and

behaved like a normal healthy dog) at which time the peritoneal cavity contained a slightly increased amount of peritoneal fluid and minute scattered granulations over parietal and visceral peritoneum and the cultures grew *Escherichia coli.*

C. All the animals in the third group (treated with 500,000 units of penicillin daily for six days) survived the acute phase of experimentally induced peritonitis. At first they were acutely ill, but soon all evidence of toxicity and illness disappeared. Exploratory laparotomies performed on the seventh postoperative day revealed evidence of subsiding peritonitis, and bacterial cultures grew a mixed bacterial flora. One animal died with fulminating acute diffuse peritonitis due to reactivation of the infection at the exploratory laparotomy on the seventh day. The other four animals were examined again at laparotomy on the 30th day when positive bacterial cultures were obtained in three dogs and all had scattered pin point size granulations throughout the peritoneum. One animal was apparently healthy for two months postoperatively, then acute diffuse peritonitis developed (pure culture of *Escherichia coli*) with extensive exudation and necrosis, and the animal died.

Two months postoperatively exploratory laparotomies on the remaining survivors still revealed minute scattered granulations, but the bacterial cultures were sterile. The results are summarized in the following table:

TABLE XV.—*Experimental Peritonitis*
Group VII. 13 animals.

Penicillin (commercial) given intramuscularly in divided doses every 4 hours				
Group	Daily Penicillin Dosage (units)	Number of Dogs	Recovered	Died
A.	100,000	3	1	2
B.	200,000	5	2	3
C.	500,000	5	5*	0

* One dog died with Escherichia coli peritonitis two months after treatment.

Table XVI shows the bacteriology in these groups of animals treated with commercial penicillin.

Table XVII shows blood and peritoneal fluid concentrations following the administration of 16,667 units of commercially available penicillin intramuscularly.

GROUP VIII. PENICILLIN AND STREPTOMYCIN THERAPY COMBINED

Five animals in which peritonitis of appendiceal origin was produced were treated with penicillin and streptomycin for six days, beginning six hours postoperatively. Each animal received 500,000 units of penicillin and 2.4 Gm. of streptomycin daily in divided doses, given at four-hour intervals by intramuscular administration. All five animals in this group survived. The findings were almost identical to those in the animals of Group VII-C which were treated with 500,000 units of penicillin daily.

TABLE XVI.—*Experimental Peritonitis*
Group VII.

Bacteriology—commercial penicillin series

Group A. 100,000 Units Daily	6 Hours (3 dogs)	7 Days (1 dog)	Necropsy (2 dogs)
Escherichia coli	3	0	2
Pseudomonas aeruginosa	0	0	1
Bacterioides	0	0	1
Clostridia	3	1	2
Alpha streptococcus	3	0	2
Beta streptococcus	1	0	2
Gamma streptococcus	1	1	1
Streptococcus fecalis	0	0	1

Group B. 200,000 Units Daily	6 Hours (5 dogs)	7 Days (2 dogs)	30 Days 1 dog)	Necropsy (4 dogs)
Escherichia coli	2	1	1	2
Clostridia	5	2	0	3
Alpha hemolytic streptococcus	0	0	0	1
Beta hemolytic streptococcus	1	0	0	1
Streptococcus fecalis	4	?	0	3
Staphylococcus albus	1	0	0	0
Staphylococcus aureus	1	0	0	0

Group C. 500,000 Units Daily	6 Hours (5 dogs)	7 Days (5 dogs)	30 Days (4 dogs)	Necropsy (60 days 1 dog)
Escherichia coli	1	5	3	1
Aerobacter aerogenes	0	3	0	0
Clostridia	2	5	2	0
Streptococcus fecalis	0	4	1	0
Beta hemolytic streptococcus	1	0	0	0
Gamma streptococcus	1	0	0	0
Staphylococcus aureus	1	0	0	0

TABLE XVII.—*Experimental Peritonitis*
Group VII.

Blood and peritoneal fluid concentrations of penicillin (units per cubic centimeter) following intramuscular injection of 16,667 units of penicillin

Hours	Blood	Peritoneal Fluid
0	0	0
1	1.20	0.62
2	2.50	1.20
3	0.62	0.31
4	0.15	0.31

TABLE XVIII.—*Experimental Peritonitis*
Group VIII. 5 animals.

Penicillin (500,000 units daily) and Streptomycin (2.4 Gm. daily) given intramuscularly in divided doses at 4 hour intervals

Organisms Cultured	Incidence 6 Hours (4 dogs)	7 Days (5 dogs)	28 Days (1 dog)
Escherichia coli	1	3	0
Clostridia	3	4	1
Streptococcus fecalis	0	3	1
Nonhemolytic *staphylococcus albus*	1	1	1
Diphtheroid bacillus	1	0	0

Table XVIII shows organisms cultured in this group of experiments.

Table XIX shows penicillin and streptomycin susceptibility of organisms cultured before therapy, and after seven days of therapy with streptomycin and penicillin.

TABLE XIX.—*Experimental Peritonitis*
Group VIII.

Bacterial susceptibility tests. Cultures made from animals receiving penicillin and streptomycin therapy (Units per cubic centimeter)

	6 Hours		7 Days	
Organisms Cultured	Penicillin	Streptomycin	Penicillin	Streptomycin
Escherichia coli	25.0	35.0	–200.0	300–600.0
Clostridia	0.57	1.05	12.5–100.0	17.25–300.0
Streptococcus fecalis	0.028	0.05	13.12– 50.0	9.3 –150.0
Nonhemolytic *staphylococcus albus*	0.57	1.05		

GROUP IX. PENICILLIN-X (INTRAMUSCULAR)

Penicillin containing 15 to 25 per cent penicillin-x was given to a group of ten animals in which experimental peritonitis of appendiceal origin had been produced. Treatment was started six hours after the surgical procedure to produce peritonitis. The penicillin-x was given intramuscularly in divided doses at four-hour intervals. A total dosage of 100,000 units a day for six days was given each animal. Nine animals survived and one expired after living 76 hours after the onset of peritonitis. All survivors recovered very rapidly from the initial acute illness on penicillin-x therapy. Recovery from toxicity and lethargy occurred very early, and normal appetite and activity were observed during the first few days of treatment in these animals.

Bacteriologic cultures made from the peritoneal fluid from the animal that died grew Escherichia coli, *Proteus vulgaris,* Bacterioides and an aerobic spore-forming Bacillus, all relatively resistant to penicillin-x. Exploratory laparotomies performed seven days postoperatively on the survivors showed evidence of a subsiding acute peritonitis, and bacterial cultures made at that time grew penicillin-resistant gram negative flora, Clostridia (3 animals) and a penicillin resistant *streptococcus fecalis* (1 animal).

Exploratory laparotomies performed 30 days later revealed no evidence of the peritonitis and all bacterial cultures made at this time were sterile.

Table XX indicates the blood and peritoneal fluid concentrations of penicillin following intramuscular administration of penicillin-x.

Table XXI gives the results and bacteriologic findings in this group of experiments.

GROUP X. PENICILLIN-X (INTRAPERITONEAL)

Five dogs in which experimental appendiceal peritonitis had been produced were treated with intraperitoneal instillations of penicillin containing 15 to 25 per cent penicillin-x, beginning six hours postoperatively. Each animal received 100,000 units of penicillin (with 15 to 25 per cent penicillin-x) twice

the first day, and 100,000 units daily thereafter for six days. Two animals survived and three died. One died during the course of therapy, one died immediately after the cessation of therapy, and the third animal died several days after the completion of therapy.

TABLE XX.—*Experimental Peritonitis*
Group IX.

Blood and peritoneal fluid concentrations of penicillin (units per cubic centimeter) following intramuscular injections of 16,667 units of penicillin containing 15 to 25 per cent penicillin-x

Hours	Blood	Peritoneal Fluid
0	0	0
1	2.50	1.20
2	2.50	0.62
3	2.50	0.62
4	0.31	0.62

Necropsy in the three fatal cases showed a diffuse peritonitis, and bacteriologic cultures grew the usual mixed flora from the colon. The animals that died were acutely ill and toxic. The two animals that survived were examined by exploratory laparotomy two weeks and four weeks postoperatively. Diffuse, extensive granulations up to ¼ inch in thickness were found throughout the peritoneal cavities of these two animals and bacterial cultures revealed persistence of the pathogenic organisms. These two animals were

TABLE XXI.—*Experimental Peritonitis*
Group IX. 10 animals.

Penicillin (15 to 25 per cent pencillin-x) intramuscularly, 100,000 units daily (16,667 units every 4 hours) for 6 days

Organisms Cultured	Incidence		
	6 Hrs. Postop. (8 dogs)	7 Days (9 dogs)	30 Days 5 dogs)
Escherichia coli	6	5	0
Proteus vulgaris	1	2	0
Aerobacter aerogenes	0	1	0
Pseudomonas aeruginosa	0	1	0
Aerobic sporeforming bacillus	0	1	0
Clostridia	8	3	0
Alpha hemolytic streptococcus	5	0	0
Beta hemolytic streptococcus	2	0	0
Streptococcus fecalis	0	1	0
Nonhemolytic *staphylococcus albus*	4	0	0
Hemolytic *staphylococcus albus*	2	0	0
Diphtheroid bacillus	1	0	0

acutely ill at the onset of the peritonitis, but signs of toxicity rapidly decreased during intraperitoneal penicillin therapy and their appearance and behavior seemed entirely normal at the time of the follow-up exploratory procedures.

Table XXII shows the results and the bacterial cultures in this group of experiments. There was no alteration of penicillin susceptibility in the bacteria during the course of intraperitoneal therapy with penicillin-x.

Table XXIII shows blood and peritoneal fluid penicillin levels in this group of experiments.

SUMMARY

Table XXIV summarizes the results of these experiments in the treatment of peritonitis of appendiceal origin in dogs with sulfonamides and antibiotics.

TABLE XXII.—*Experimental Peritonitis*
Group X. 5 animals.

Penicillin (15 to 25 per cent penicillin-x) 100,000 units intraperitoneally daily for 6 days

Organisms Cultured	Incidence			
	6 Hours Postop. (8 dogs)	Necropsy (3 dogs)	15 Days (2 dogs)	30 Days (2 dogs)
Escherichia coli	5	3	2	2
Proteus vulgaris	2	1	0	0
Clostridia	5	3	2	2
Alpha hemolytic streptococcus	3	2	0	2
Beta hemolytic streptococcus	2	2	2	1
Gamma streptococcus	1	0	0	0
Streptococcus fecalis	1	0	1	0
Nonhemolytic *staphylococcus albus*	1	0	2	0
Hemolytic *staphylococcus albus*	2	2	0	0
Hemolytic *staphylococcus aureus*	2	0	0	0
Diphtheroid bacillus	1	0	0	0

TABLE XXIII.—*Experimental Peritonitis*
Group X.

Blood and peritoneal fluid concentrations of penicillin (units per cubic centimeter) following the intraperitoneal instillation of 100,000 units of penicillin (15 to 25 per cent penicillin-x)

Hours	Blood	Peritoneal Fluid
0	0	0
1	19.96	80.00
2	9.95	80.00
3	4.99	9.95
4	1.20	4.99
10	.....	0

CONCLUSION

Fulminating diffuse peritonitis was produced in 93 dogs by dividing the vascular supply to the appendix; ligating the base, and crushing the appendix. The omentum and spleen were removed. Twenty untreated control animals died with acute diffuse peritonitis from bacterial infection with intestinal organisms. The average survival period was 39 hours.

Sulfonamide therapy with (1) intravenous sodium sulfadiazine (5 dogs), (2) intraperitoneal sulfasuxidine (5 dogs), and (3) combined intraperitoneal sulfanilamide and intravenous sodium sulfadiazine (5 dogs) apparently had no beneficial effect. All died with peritonitis similar to that observed in the control animals. However, the survival period in the sulfanilamide-sulfadiazine group was prolonged to 80 hours.

Streptomycin therapy given intramuscularly (10 dogs) and intraperitoneally (5 dogs) apparently prolonged the survival period of dogs with experimental appendiceal peritonitis to averages of 75 to 92 hours in 14 out of 15 dogs. One animal survived. However, doses of streptomycin that effectively controlled the organisms in the peritoneal cavity caused death from the toxic effect of streptomycin (apparently on the medullary respiratory center).

Commercially available penicillin given intramuscularly at four-hour intervals daily in doses of 100,000 units (3 dogs) 200,000 units (5 dogs) 500,000 units (5 dogs), and 500,000 units combined with streptomycin 2.4 Gm. was definitely beneficial in the treatment of experimental appendiceal peritonitis. All animals receiving 500,000 units of penicillin daily survived.

TABLE XXIV.—*Comparison of Therapeutic Agents for the Treatment of Experimental Peritonitis of Appendiceal Origin.*

	*Treatment	Number of Dogs	Recovered	Died	Average Survival Hours
	Controls—untreated	20	0	20	39
1.	Sulfadiazine 4 Gm. twice daily intravenously	5	0	5	44
2.	Sulfadiazine 4 Gm. twice daily intravenously and Sulfanilamide 5 Gm. intraperitoneally	5	0	5	80
3.	Sulfasuxidine intraperitoneally 1.0 Gm. per kilogram of body weight daily	5	0	5	40
4.	Streptomycin intramuscularly, 2.0 Gm. daily (started 6 hours postoperatively)	10	1	9	92
5.	Streptomycin intramuscularly, 2.0 Gm. daily (started immediately postoperatively)	5	0	5	75
6-A.	Streptomycin intraperitoneally 0.4 to 1.6 Gm. daily	5	0	5	87
6-B.	Streptomycin intraperitoneally 4.0 Gm. daily	5	0	5	12
7-A.	Penicillin intramuscularly 100,000 units daily	3	1	2	37
7-B.	Penicillin intramuscularly 200,000 units daily	5	2	3	65
7-C.	Penicillin intramuscularly 500,000 units daily	5	5	0	..
8.	Penicillin intramuscularly 500,000 units daily, and Streptomycin intramuscularly 2.4 Gm. daily	5	5	0	..
9.	Penicillin-x (15 to 25 per cent) intramuscularly 100,000 units daily	10	9	1	76
10.	Penicillin-x (15 to 25 per cent) intraperitoneally 100,000 units daily	5	2	3	135

* All therapy was begun 6 hours postoperatively unless otherwise specifically designated. All intramuscular therapy was given in equally divided doses at four-hour intervals.

Penicillin containing 15 to 25 per cent penicillin-x in doses of 100,000 units daily intramuscularly (10 dogs), and intraperitoneally (5 dogs) was effective in the treatment of experimental appendiceal peritonitis. Nine out of 10 animals treated intramuscularly, and 2 out of 5 animals treated intraperitoneally with penicillin-x recovered.

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