THE VALUE OF CERTAIN THERAPEUTIC AGENTS AGAINST THE BACTERIAL INFECTIONS CAUSING CONJUNCTIVITIS AND RHINITIS IN DOGS AFFECTED WITH DISTEMPER

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In memory of my brother

Colonel L. C. Hsiung

and his wife

Mrs. Rosetta Ling Hsiung

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INTRODUCTION

Canine distemper is a highly infectious disease of dogs. The cause of this disease was first discovered to be a filtrable virus by Carré in 1905. Young dogs under one year of age are easily infected. During the course of the virus infection covering a period of five to twelve days, the dog's resistance becomes so low that it cannot fight off bacterial invasion. The germs which are present in the bacterial phase of the disease are secondary invaders and cause most of the symptoms.

As a result of bacterial findings in canine distemper, Ferry (1910) came to the conclusion that the ocular, nasal, cutaneous, and nervous symptoms are the result of secondary infection, and death in most cases results from these secondary invaders. If this is true, the distemper vaccine will have little or no direct effect.

At the seventieth meeting of the American Veterinary Medical Association, Dr. E. A. Cahill (1933) stated during a discussion "Regardless of whether the filtrable virus is the primary cause and Bacillus bronchisepticus a secondary invader of distemper or vice versa, there is increasing evidence throughout the country of a very great need on the part of the practitioner for something besides the regular treatment for canine distemper.... There is increasing evidence that the antibronchisepticus serum and the antifiltrable virus serum are not always one hundred percent successful...."

The prevalence and methods of treatment of canine distemper have long been problems of economic importance to the veterinarian and the dog owner. Many vaccines and sera have been used for the treatment of canine distemper, but published data on local application of therapeutic agents against secondary invaders of the eyes and nose are quite limited. The object of this study was to determine the efficacy of the various drugs that are applied locally against secondary infection in dog distemper.

HISTORICAL REVIEW

I. Bacteriological Studies on Canine Distemper

Canine distemper is said to have been known in the time of Aristotle. One of the first to advance a definite conception of the nature of the disease was Jenner (1815). He recognized the contagious character of the disease, and noted that the causative agent retained its infectious properties for a long time after separation from sick dogs. Jenner was the first to differentiate between distemper and rabies.

Early studies on the microbiology of canine distemper were directed at the discovery of the primary cause of the disease. A number of different organisms were found, and results could not be confirmed; therefore the question of etiology was not definitely established until 1905, when Carré discovered the filtrable virus of distemper.

The first bacteriological investigation of canine distemper was by Semmer (1875). He found a small and exceedingly slender bacillus in the blood of diseased dogs a few hours after they died of distemper. Krajewski (1881) found a micrococcus. Marcone and Meloni (1904) found cocci similar to staphylococci.

Rabe (1883) and Mathis (1887) cultivated streptococci and staphylococci from the pustular contents, nasal exudate, the conjunctival secretion, the blood and various organs. They claimed that these organisms were specifically related to distemper.

Millais (1890) found a long bacillus which liquefied gelatin, descending as a flaky mass in the almost clear fluid which became covered by a whitish scum. The worker also found a micrococcus which was thought to be the cause of lung lesions.

Galli-Valerio (1896) found a bacillus on agar plates, from the mucus of the respiratory tract. It ranged in size from 0.3 x 1.2 to 0.3 x 2.5 microns. It was often dumbbell shaped. The bacillus was gram-positive and was motile. This organism was also isolated from the lungs, brain, spinal cord and pus from the frontal sinus.

Taty and Jacquin (1898) found a diplococcus in the central nervous system which they regarded as the cause of the nervous form of distemper.

Jess (1899) isolated an ovoid bipolar-staining bacillus from the nasal discharge, blood, and conjunctival secretion of distemper dogs.

Copeman (1900) found a cocco-bacillus in smears from broth, not infrequently in chains and sometimes of considerable length. It was gram-negative, but grew readily on agar at 36°C.

Lignieres (1903) isolated and described an organism which was also studied by Phisalix (1903). They obtained from the blood of the heart and internal organs an organism in the form of a long bacillus, which, after passage through guinea pigs or on cultures, soon changed into a short coccobacillus. To this organism was given the name Pasteurella canis.

Carré (1905) claimed that canine distemper was caused primarily by a filtrable virus, and that the disease as a whole consisted of a series of progressive secondary infections caused by a number of cultivable bacteria.

Hewer (1906) stated that cocci which gave the reactions of the pyogenic staphylococci were obtained in pure culture from the nose and bronchi. Staphylococcus albus was most frequently found, although Staphylococcus aureus was often present. He isolated several bacteria from a few cases of distemper, but could not prove their importance.

Ferry (1910) described a microorganism which, in 1911, he named Bacillus bronchicanis. This organism differed from the organisms described by Galli-Valerio (1896), Copeman (1900), Lignieres (1903), Phisalix (1903) and Hewer (1906). Ferry (1910) found that when cultures were taken early in the disease, B. bronchicanis was found in the respiratory tract in every case. If cultures were taken in the first stage of distemper, they were uncontaminated. Purulent discharges from the eyes and nose were due to secondary infections and were not true manifestations of distemper. He concluded that B. bronchicanis was the primary and essential etiological factor in canine distemper.

McGowan (1911), at the Royal College of Physicians, Edinburgh, Scotland, reported the same organism as that described by Ferry. He isolated this bacillus without difficulty from the mucopurulent nasal discharge from the trachea or lungs, but not from the blood. This organism, when applied to nasal mucous membranes, produced the clinical symptoms of distemper. In one of the experimental dogs, the nose was absolutely plugged with pus on the fifteenth day after inoculation, and cultures from the nose taken then gave staphylococci only. Staphylococci and also bacilli were shown in large numbers in cultures from the nose on the sixteenth day after inoculation.

Torrey and Rahe (1913) obtained cultures from the eyes by streaking directly on agar plates. The cultures from nasal exudate were emulsified in sterile solution and plated. Their bacterial findings were as follows:

1. During incubation period - Streptococcus fecalis was found in the eyes. Bacillus aerogenes, Bacillus bronchisepticus, Streptococcus pyogenes, Albococcus epidermidis, Pasteurella canis, Bacillus coli, and Strep. fecalis were found in the nose.
2. During first week of symptoms - Streptococci and Albo. epidermidis were found in the eyes. B. bronchisepticus, Albo. epidermidis and Aurococcus aureus were found in the nose.
3. During second week of symptoms - Gram-positive diplococci, Albo. epidermidis and a few streptococci were found in the eyes. Streptococci, albococci, and B. bronchisepticus were found in the nose.

4. Chronic cases, three or more weeks - The same results were obtained as above.
5. Recovered cases - Very few organisms were found in the eyes and they were the same as above.
6. Fatal cases - Only one out of five cases yielded Albo. epidermidis and Strep. fecalis. Streptococci, albococci and a few colonies of B. bronchisepticus were found on plates from the nasal exudate.

In summary, among the different organisms isolated, two percent of the cultures from the eyes and 56 percent of the cultures from the nose were B. bronchisepticus. They concluded that B. bronchisepticus was the infective agent of the disease, but certain symptoms such as those of the eyes and nose might be due wholly or in part to secondary infection. Streptococci, B. coli and Bacillus enteritidis were the most frequently encountered secondary invaders. Of these, the most important, as far as the clinical picture and the severity of the disease was concerned, were the streptococci. It was their observation that the secondary invaders began to multiply in the tissue only after the animal had become exhausted by the toxin of the bacilli.

Ferry (1912a, 1912b) reported that B. bronchicanis may be the cause of a severe infection among laboratory animals other than dogs. He changed the name of the microorganism from B. bronchicanis to B. bronchisepticus. Taking into consideration the combined results of McGowan (1911), Torrey and Rahe (1913), and himself, he concluded that the condition known as distemper in the dog, and certain other animals, was an acute infectious disease due to B. bronchisepticus. This organism produced a catarrhal inflammation, primarily of the larynx and trachea, and possibly of the nasal cavity. The infection often extended to other mucous surfaces, resulting in general infection, followed by many complications and sequelae due to secondary infections. The mortality rate was from 60 to 90 percent. The investigator (1913, 1914) strongly suggested that suspensions of both live and killed B. bronchisepticus would protect dogs from natural distemper.

Schoichi (1932) stated in his bacteriological studies of distemper that 50 percent of the dogs showed pure cultures of B. bronchisepticus which in 35 percent of the animals was associated with streptococci, staphylococci and even B. coli.

Lockhart, Ray and Barbee (1925) published the results of their work which showed that a true virucidal serum could be prepared in dogs hyperimmunized against the filtrable virus of Carré. They concluded that this serum was a reliable immunizing agent, producing an immunity of long duration after injection into dogs. They were the first to make a useful antidistemper serum.

Dunkin and Laidlaw (1926a, 1926b) reported a filtrable virus as the cause of canine distemper and concluded that B. bronchisepticus was only a secondary invader. Research on distemper virus vaccine was conducted by them. In the same year Pugh (1926) stated that distemper is due to a filter-passing virus and regarded B.

bronchisepticus as a secondary invader.

Lockhart (1927) stated that true distemper is a systemic disease, and that the things which are usually considered visible symptoms are secondary in character, being produced by organisms which are ordinarily of low virulence. These are capable of producing disturbances in devitalized tissue. The bacteria recoverable from distemper cases vary greatly, but generally Alcaligenes bronchisepticus, Staphylococcus and Streptococcus are found.

Schlingman (1931) advanced the possibility of a hemolytic streptococcus being associated with canine distemper. Good results in the treatment of distemper were obtained from the use of an antiserum and a mixed bacterin. He (1932) reported on the bacteriological studies of canine distemper in one hundred naturally infected cases. From the different organs such as the lower trachea, lungs, liver, spleen and heart blood he isolated B. bronchisepticus from 81 percent of the animals, streptococci from nine percent, Staph. albus from six percent and colon-typhoids from four percent. Staphylococcus aureus and Staph. citreus were seldom present. They should be considered as secondary invaders.

Pyle (1934) reported a bacteriological examination of 146 spleens, taken from distemper infected puppies late in the filtrable virus stage of the disease. At this time there was a second rise in temperature, reaching a high level simultaneously with the appearance of the characteristic distemper conjunctivitis and rhinitis. Salmonella enteritidis was isolated from 20 spleens. An unknown micrococcus and Staphylococcus albus were isolated from one spleen each. The remaining 124 spleens were bacteriologically sterile. Two of the 13 spleens from distemper infected adult dogs showed Sal. enteritidis, whereas 21 spleens from distemper infected ferrets all proved to be sterile. In no instance was Al. bronchisepticus isolated from any of 180 spleens examined.

Regenos (1935) stated in his comprehensive studies on canine distemper: "In canine distemper, there is no question but that the filtrable virus is the usual primary causative agent and that the following bacterial organisms should be considered as having etiological significance: Al. bronchisepticus, streptococci, Sal. paratyphosus B, Sal. enteritidis, and staphylococci. The occurrence and importance will vary with the seasons and localities. In general, the importance of the organisms is in the order given." Similar statements were made by Whitney (1940).

Greene (1943) and Schlotthauer (1949) claimed that the cause of canine distemper is a specific filtrable virus. The disease is always complicated by numerous secondary invaders, e.g. Sal. enteritidis, Brucella bronchiseptica, various staphylococci and streptococci.

Hsiung, Eads and Stafseth (1950) found that Micrococcus pyogenes var. albus and species of streptococci were the commonest organisms found in serous and mucopurulent ocular discharges from dogs affected with distemper.

II. Therapeutic Agents

Adler (1937) found that successful results were obtained with polyvalent antiserum in cases of distemper in dogs and cats, complicated with suppurative keratitis, caused by such microorganisms as streptococci, staphylococci, pneumococci and other common pyogenic bacteria. This treatment hastened the local and general antibacterial responses, both humoral and cellular.

Greene (1943) claimed that the secondary infections in canine distemper may be treated with one of the sulfonamides. Ophthalmic ointments and nasal solutions should be used as supplemental treatment besides the antidistemper serum and mixed infection serum.

The control of canine distemper is largely dependent upon the use of biologics. Homologous antidistemper serum (canine) has been used extensively for the last few decades. Schlotthauer (1949) reported that the efficiency of this antiserum for treatment varied with the state of virulence of the virus that was present in the animal body.

In 1950, Eads stated: "The aim in treating distemper-affected dogs is to promote everything that tends to conserve the energy and vitality of the subjects. It is, therefore, essential that the patients should be kept as comfortable as possible."

Sulfa Drugs

The use of sulfa drugs is effective in reducing the mortality due to distemper in dogs and has been reported by many workers.

Marcus and Necheles (1938) demonstrated in their work that sulfanilamide and prontosil could be used successfully in the treatment of distemper in dogs, since in most of the fatal cases, streptococci, staphylococci and B. bronchisepticus were more fatal than the virus itself.

Bryan (1941) reported that sulfapyridine was apparently a specific therapeutic agent in the treatment of canine distemper in the early stages of the disease. The combined use of sulfapyridine and homologous anticanine distemper serum was even more effective.

Guyton (1941) reported that sulfanilamide in ointment form was a suitable preparation for local use. In eighteen cases of catarrhal conjunctivitis, due to ordinary infectious organisms, such as staphylococci and streptococci, five percent sulfanilamide ointment gave encouraging results. He suggested that sulfathiazole might prove to be better when used locally for certain types of infection.

Richtner (1942) claimed that the bacteria disappeared rapidly from the nose upon local treatment with sulfathiazole in certain acute inflammatory conditions.

Thygeson and Braley (1943) found that the use of five percent sulfathiazole ointment was effective in the treatment of chronic conjunctivitis caused by staphylococci. They also found that in cases in which the Morax Axenfeld diplobacillus was present, treatment with zinc sulfate was useless until the staphylococci were eliminated.

Alvaro (1945) reported from Brazil that local sulfonamide therapy was very effective in a number of well-defined eye diseases. Since sulfonamides have only bacteriostatic action, it is essential that the drug be applied frequently. Repeated instillations and applications of suitable ointment appeared to be the method of choice. He also stated that the sulfonamides are almost innocuous to the ocular tissues when applied locally, and he recommended the local use of sulfonamides because of easy penetration and tolerance.

Robson and Scott (1942) suggested that the local application of certain sulfonamides might be of value in the treatment of infective conditions of the eye. According to their experimental results, 30 percent sodium sulfacetamide had produced no irritation or other ill effects. In 1943 these workers also claimed that a 30 percent solution of sodium sulfacetamide gave the same results as penicillin, by which Staph. aureus was eliminated from the flora of the conjunctival sac. Fifteen percent solution of solubilized sulfathiazole was less effective, and 2.5 percent sodium sulfacetamide was of little or no value.

Cortes (1947) reported that cases of acute and purulent conjunctivitis responded to sulfacetamide. The drug was administered locally and systemically.

Kuhn (1947), in Scotland, found that neither a solution nor an ointment of 30 percent sodium sulfacetamide was irritating and no allergic reactions occurred. He used a drop of the solution every four hours for three days after the removal of a foreign body.

Benedict and Henderson (1947) demonstrated that a 30 percent sodium sulfacetamide gave the best results in average cases of acute catarrhal conjunctivitis and acute conjunctivitis associated with purulent or mucopurulent discharges.

Leopold (1948) reviewed the merits of sulfonamide drugs for local use. He investigated the penetration into the anterior chamber of the eye by most available sulfonamide compounds. The concentration of the drug in the aqueous humor was determined in normal eyes and eyes in which the cornea had been damaged. The drugs in various concentrations were applied as drops with and without detergents and in various ointment bases. He concluded that the penetration of locally applied sulfonamides depends on the physical form of the compound, its solubility, the vehicle, the presence of a detergent and the state of the cornea. Of the preparations available, sodium sulfacetamide, sulfadiazine, and sulfapyridine would appear to be the drugs of choice, in the order mentioned.

Eads (1949) found that sulfamerazine was very useful in the treatment of a variety of conditions, commonly found in small animal practice, namely, distemper and respiratory infections. He stated that the use of sulfamerazine for infections due to bacteria associated with clinical distemper and respiratory infections such as bronchitis, rhinitis, laryngitis, etc., was of definite value in his study.

Bacitracin

Johnson, Meleney and Auker (1945, 1947) have shown that, in general, bacitracin is effective against the same bacteria as penicillin, and, in addition, the organisms are often more susceptible to bacitracin than to penicillin, in a ratio of five to one. In one hundred cases of surgical infections treated locally with bacitracin, favorable response was evident in 88 percent of the patients.

Bellows and Farmer (1948a, 1948b) reported that a bacitracin-sensitive hemolytic Staph. aureus infection can be prevented when treated with bacitracin within a definite time interval in experimental eye infections. Good results were obtained in acute infections in clinical cases of conjunctivitis that had been treated with bacitracin.

Miller, Slatkin, and Johnson (1949) reported that 500 units per gram of bacitracin, effective against gram-positive organisms, were incorporated into several ointment bases. Superiority of bacitracin over sulfonamides and penicillin rests in the low rate of sensitization of the patient. Thus far only the 0.5 percent solution was found to produce sensitivity.

Streptomycin

In 1944 streptomycin was shown by Schatz et al. in *in vivo* and *in vitro* experiments to be bacteriostatic against certain gram-positive organisms as well as a wide variety of gram-negative forms.

Robinson, Graessie and Smith (1945) found that streptomycin was active *in vitro* against a variety of gram-negative and gram-positive bacteria. The former included *Eberthella*, *Salmonella*, *Escherichia*, *Shigella*, *Klebsiella*, *Brucella* and *Proteus*. The gram-positive organisms were strains of *Strep. hemolyticus*, *Staph. aureus* and *Diplococcus pneumoniae*.

Owens (1946) reported one case of a severe corneal infection caused by *Esch. coli*, which responded satisfactorily to local application of streptomycin.

Alberstadt and Price (1946) treated nine patients for corneal infections with streptomycin applied locally. In spite of the fact that adequate bacteriological data were unobtainable, they concluded that the addition of this antibiotic to the usual form of treatment definitely shortened the healing time.

Leopold and Nichols (1946, 1949) reported that the local use of streptomycin gave the same result as penicillin, though the spectrum of its activity was not the same as the latter.

Bellows, Burkholder, and Farmer (1947) demonstrated that experimental corneal ulcers, produced by injection of *Bacillus pyocyaneus*, were prevented by applications of saline solution containing 10,000 mcg. per ml. of streptomycin. In the same year, Bellows and Farmer (1947a) found that in acute and chronic conjunctivitis, where known organisms were present before treatment with streptomycin, the sac became sterile after a few days of instillation therapy. Healing generally was prompt if complicating factors were absent. Furthermore, these investigators (1947b) reported that streptomycin is safe and non-irritating to the surface of the eyeball in concentrations up to 10,000 S units per ml. Local application of streptomycin decreases the amount of secondary infections accompanying vaccinia infections of the cornea.

Kellberg (1947) found that streptomycin's main effectiveness has been in conquering those very persistent, lowgrade bacillary infections that commonly complicate distemper in dogs.

Frenken (1948) reported that some surprising results were obtained in the local treatment of purulent rhinitis with streptomycin. A solution of one gram of streptomycin in 30 ml. of saline solution was recommended to be given on three consecutive days, three times daily, in doses of two and half to three ml. No relapses occurred during the period of four months' observation.

Grignolo (1948) reported that in 72 patients, streptomycin was administered in several ways into the eye. No improvement was noted as result of treatment of corneal ulcerations due to pneumococci, staphylococci or streptococci. Streptomycin appeared to be effective only against B. coli, Bacillus friedlander, Bacillus proteus and a few other organisms.

Lepri (1950) found that 50,000 units of streptomycin injected subconjunctivally in rabbits showed the same manner of diffusion as penicillin, but at a much slower rate.

Eads (1951b) reported that streptomycin showed little, if any, value in the treatment of canine distemper even when administered at the rate of 11,000 S units per pound of body weight, four times per day for ten days.

Penicillin

Penicillin in a valuable drug in the treatment of some infections. It is selective in its antibiotic action and it is not effective in all infections. Most of the organisms which respond favorably to penicillin therapy are gram-positive.

Abraham, Chain, Fletcher, Gardner, Heatley, Jennings and Florey (1941) demonstrated in four cases that the local application of penicillin to the human eye resulted in rapid relief from pain and resolution of the inflammation. Swabs from the eye of one patient revealed Staph. aureus.

Robson and Scott (1943) found that penicillin gave a definite beneficial reaction subsequent to local application in the eyes. Staph. aureus was the microbe eliminated from the conjunctival sac. When treatment was begun. 24 hours after inoculation of the organisms, little or no benefit was produced by the application of penicillin. The importance of early treatment and repeated applications in clinical use of this drug should be emphasized.

Riser (1945) stated that penicillin is bacteriostatic rather than bactericidal in its curative action. It has been used in aqueous solutions for injections, solid tablets for oral administrations, and ointment for local applications.

Leopold and La Motte (1945) showed that the penetration of penicillin into the eye is greatly enhanced in the presence of infections or abrasions of the cornea. The dramatic cures that have resulted from the use of penicillin have led to rather indiscriminate use of the drug.

Penicillin therapy aimed at the secondary invaders in canine distemper has been reported by Davidson (1945). In a single complicated case of canine distemper, he obtained recovery in five days, using 10,000 Oxford units of penicillin intravenously and intramuscularly. The purulent discharge from the eyes had decreased greatly in amount after 24 hours from the first injection. Only a scanty discharge appeared after the second injection and a small amount of catarrhal exudate after the third treatment.

Costi and Alvarez (1947) reported good results by the local use of crystalline penicillin in acute, subacute and chronic conjunctivitis.

Garcia (1947) worked on the concentration of penicillin in various ocular tissues following various methods of administration. He found that penicillin reaches the highest concentration in the tissue when it is given locally. Good results were obtained in the treatment of conjunctivitis.

Penicillin was used locally in a total of 153 cases by Bitran (1947). He concluded that the drug should be used locally in ophthalmology and not systemically.

Sorsby and Ungar (1946) and Minton (1946) reported that pure penicillin is well tolerated by the eye when applied locally in ointment containing up to 100,000 units per gram.

Micuda and Holt (1947) reported on the use of penicillin in canine distemper meningitis. Dramatic results were obtained when intraspinal injection of penicillin in saline solution was used.

Collins (1948) in his studies on penicillin in veterinary medicine, came to the conclusion that, although the canine distemper virus is not amenable to penicillin activity, the relative sensitivity of the usual secondary invaders associated with the virus-caused disease, indicated the use of the substance for the treatment of the secondary complications.

Holstege (1950) claimed that penicillin salve was a valuable agent in the treatment of external diseases of the eye, and when it was combined with a sulfonamide, its range of indication extended to almost all infectious external diseases of the eye.

Lugossy (1950) enumerated various infectious diseases of the eyelid, orbit and globe, in which penicillin should be given either by local application, or by the subconjunctival or intramuscular route. He stated that penicillin should be given for two additional days after a clinical cure had been noted, in order to prevent a remission.

Hsiung, Eads and Stafseth (1950) found that calcium penicillin ointment was highly bacteriostatic to organisms found in either serous or mucopurulent discharges from the conjunctiva. There was a marked decrease in the number of bacterial colonies present four hours following the application of the penicillin ointment.

Eads (1951b) reported that amorphous penicillin at the rate of 1,000 to 1,500 units per pound of body weight, given from four to six times daily, was an effective agent in controlling the secondary invaders of distemper. He (1951a) also stated that he liked the use of penicillin ointment for local application.

III. Serological Studies on Identification of Organisms

Van de Velde (1898) first demonstrated specific agglutination between a univalent serum and its homologous streptococcus. Later Kinsella and Swift (1917, 1918a, 1918b) reported that the classification of hemolytic and non-hemolytic streptococci was determined by the complement fixation reactions between the organisms and their antisera.

Dochez, Avery and Lancefield (1919) studied the biology of streptococci. Antigenic relationships among strains of *Streptococcus hemolyticus* were demonstrated by them. Four biological types of streptococci were identified by means of the agglutination reactions and protection.

Avery and Heidelberger (1923, 1925) found that pneumococci can be distinguished readily one from another serologically due to the antigenic composition of the capsules.

The antigenic complexity of streptococci was studied extensively by Lancefield (1925, 1928, 1933). In 1933 she claimed that hemolytic streptococci can be differentiated serologically. She classified 106 strains of streptococci, isolated from man, animals, milk and cheese, into five groups by means of precipitation reactions. The antisera for the precipitation tests were prepared by injection of heat killed cells intravenously into rabbits.

The application of the serological method in the differentiation of strains of organisms was demonstrated by Hucker (1932). He attempted to utilize the agglutination reaction in the separation of the genera Leuconostoc and Streptococcus. He concluded that these species showed evidence of a large amount of strain specificity.

Stockinger and Carpenter (1944) studied the differences in cross reactivity among the species of Neisseria and indicated that there was an immunological relationship between certain strains.

The application of serology in the differentiation of strains of Leuconostoc mesenteroides was reported by Alvaro and McCleskey (1947). In their studies, both precipitation and agglutination methods were employed. Of these two methods, the agglutination test was the most useful in showing type relationship.

MATERIALS AND METHODS

The investigations leading to the present report were begun in June 1948 with the purpose of determining the effectiveness of various therapeutic agents on the growth of the bacteria in the conjunctival sac and nasal cavity in dogs affected with distemper.

I. Bacterial Florae in Eyes and Nose

For the purpose of checking the variety of organisms present in the eyes and nose of dogs without distemper, 22 animals were used. Fourteen dogs infected with distemper were chosen for comparison. Swabs were taken along the conjunctivae and nostrils of different dogs and sent to the laboratory immediately with a record as follows:

Sample No.	Case No.	Date
Owner		
Address		
Breed	Age	Sex
Clinical diagnosis		
Medication		
Material submitted		

These swabs were streaked directly on blood agar plates, and then placed in tryptose broth or semisolid brain-heart infusion. After 24-hour incubation at 37°C discrete colonies with different characteristics were transferred to tryptose agar slants in order to obtain pure cultures for identification. Blood agar slants were used for those organisms which failed to grow on the tryptose agar slants. One loopful of the 24-hour broth culture or the semisolid brain-heart infusion was streaked on another blood agar plate to detect any organism that was absent on the previous culture.

The morphology of the organisms from the slants was studied in Gram stain preparations. Then the organisms were placed into three groups, namely, gram-positive cocci, gram-negative cocci, and gram-negative rods. Identification and classification were based on biochemical reactions according to Bergey's Manual of Determinative Bacteriology (1948). Basic media used for the preliminary studies included fermentation broths, nitrate peptone broth, litmus milk and gelatin, which were prepared as follows:

1. Fermentation broth base

Tryptose	1	gm.
NaCl	0.5	"
Andrade's indicator	1	ml.
Distilled water	100	"

Andrade's indicator was prepared by adding 12-17 ml. of 1 N NaOH into 100 ml. of 0.2% aqueous acid fuchsin.

2. Nitrate peptone broth

Peptone	1	gm.
KNO_3	0.2	"
Dextrose	5	"
NaCl	5	"
Distilled water	1000	ml.

Nitrate test reagent

Solution A

Sulphanilic acid	8	gm.
Glacial acetic acid	250	ml.
Distilled water	750	"

Solution B

α naphthylamine	5	gm.
Glacial acetic acid	250	ml.
Distilled water	750	"

Equal amounts of solution A and B were added to the culture. A red color indicated a positive reaction.

3. Litmus milk

Litmus	2	gm.
Skim milk	1000	ml.

This medium was sterilized at 10 lbs. for 15 minutes.

4. Gelatin for stab culture (Difco dehydrated)

Bacto-beef extract	3	gm.
Bacto-peptone	5	"
Bacto-gelatin	120	"
Distilled water	1000	ml.

Special media were used for further studies according to the different groups.

A. Gram-positive cocci with heavy growth on the tryptose agar slants were grouped as Micrococcus and the following media were inoculated: Nitrate peptone broth, litmus milk, gelatin, mannitol fermentation broth, one percent, and ammonium phosphate agar. The color of the colony of the organism was always recorded for the sake of identification.

Ammonium phosphate agar

Washed agar	1.5	gm.
Ammonium phosphate	0.1	"
Glucose	1	"
KCl	0.02	"
$MgSO_4$	0.02	"
Distilled water	100	ml.

One ml. of 1.6 percent alcoholic brom-cresol purple was added to 1000 ml. of the above medium for the indicator. When the organisms utilized ammonium phosphate as sole source of nitrogen, the medium changed from purple to yellow.

B. Gram-positive cocci with pin-point colonies and fine growth on tryptose agar or blood agar slants were streaked on blood agar plates. According to their hemolytic characteristics they were subdivided into hemolytic, viridans and nonhemolytic streptococci. The hemolytic streptococci were inoculated into the following media: tryptose broth, 6.5 percent sodium chloride, sodium hippurate broth, litmus milk and fermentation broths (lactose, mannitol, glycerol, sorbitol, and trehalose, all one percent).

Tryptose broth

Tryptose	2	gm.
Dextrose	1	"
NaCl	0.5	"
Distilled water	100	ml.

This medium was used for the determination of the growth when the cultures were incubated at 45°C and 10°C.

Sodium hippurate broth

Tryptose broth	100	ml.
Sodium hippurate	1	gm.

This medium was tubed in four ml.

Benzoic acid determination

Sodium hippurate broth cultures were incubated at 37°C for four to five days. The clear broth above the growth was decanted into a second tube. This broth was acidified by adding one drop of concentrated sulfuric acid. Then the acid broth was extracted with two to five ml. of ether, and decanted into a third tube. Two ml. of Zwicker's solution was added to the ether extract. The needle crystals at the interface meant benzoic acid, therefore hippurate was split by the organisms.

Zwicker's solution

$CuSO_4$ (10 percent)	40	ml.
Pyridine	10	"
Distilled water	50	"

C. Gram-negative bacilli were inoculated into the following media: Lactose motility medium, Kligler iron agar slant, fermentation broth (dextrose, lactose, maltose, mannitol, and sucrose, all one percent), indol medium, citrate agar slants and methyl red Voges Proskauer medium. Gelatin stab cultures and litmus milk were used specially for the identification of Brucella bronchiseptica.

Lactose motility medium

Motility test medium (Difco)	1.8	gm.
Beef extract (Difco)	0.2	"
K_2HPO_4	1.0	"
Lactose	1.0	"
Andrade's indicator	1.0	ml.
Distilled water	100	"

Indol medium

Tryptone	1.0	"
NaCl	0.5	"
Distilled water	100	ml.

Indol test reagent

Paradimethylaminobenzaldehyde	5.0	gm.
Amyl alcohol	75	ml.
HCl (concentrate c.p.)	25	"

A red color indicated the positive reaction.

Voges Proskauer test reagent

Solution A

α naphthol	5.0	gm.
Alcohol (95 percent)	100	ml.

Solution B

KOH	40	gm.
Distilled water	100	ml.

A 1.2 ml. of solution A was added to two ml. culture which was incubated at 37°C for 24 hours. Then 0.4 ml. of solution B was added to the same tube. A pink or red layer indicated the development of acetylmethylcarbinol after 10 to 20 minutes.

For the methyl red test the culture was incubated for 48 hours at 37°C. Three drops of .02 percent methyl red were added to five ml. of culture. A red color indicated the presence of acid.

D. Gram-negative cocci were streaked on blood plates for the oxidase test. A one percent para-aminodimethyl-aniline monohydrochloride solution was poured on the incubated plates and poured off again immediately. Colonies of bacteria forming indophenol oxidase turned pink, changing to maroon and finally black. The following media were inoculated for the identification of Neisseria: Fermentation broths (dextrose, lactose, maltose, mannitol and sucrose, all one percent), nitrate peptone broth, litmus milk, gelatin stab, and indol medium.

II. Bacteriological Studies on the Effects of Treatment

Dogs with a clinical diagnosis of canine distemper in the veterinary hospital at Michigan State College and showing evidence of conjunctivitis or rhinitis with either a serous or mucopurulent discharges were selected for this study. These patients ranged in age from five months to three years. The animals were maintained in the comfortable kennels at the hospital during the period of treatment. Daily clinical observations were recorded.

Prior to the application of the various treatments, swabs were taken of the conjunctivae and nostrils of the dogs. These swabs were handled as previously described (Part I). Immediately thereafter one of the therapeutic agents was applied and distributed over the corneas, conjunctivae and nasal cavity.

At specified intervals after the drug had been administered such as 4, 24, 48, 72, 96, and 144 hours, swabs were taken and cultured as indicated above. In some cases swabs were taken after one week of treatment to determine the degree of bacteriostatic activity of the compound.

Eight different kinds of therapeutic agents were used and were supplied through the courtesy of the following companies:

1. Distemper serum (mixture of antiviral and antibacterial sera): Pitman Moore Company, Indianapolis, Indiana.
2. Sulfathiazole ointment (five percent): Jen-Sal Laboratory, Kansas City, Missouri.
3. Sodium sulfacetamide solution (30 percent): Schering Corporation, Bloomfield, New Jersey.
4. Baciguent ointment (500 units of bacitracin per gram): Upjohn Company, Kalamazoo, Michigan.
5. Bacitracin ointment (500 units per gram): Upjohn Company, Kalamazoo, Michigan.
6. Streptomycin solution (100,000 S units per ml.): Merck and Company, Rahway, New Jersey.
7. Potassium penicillin ointment (28,600 units per gram): Upjohn Company, Kalamazoo, Michigan.
8. Calcium penicillin ointment (14,300 units per gram): Parke, Davis and Company, Detroit, Michigan.

III. Serological Studies on Some Strains of the Isolated Organisms

Application of serology in the differentiation of three strains of *Neisseria catarrhalis* and two strains of *Micrococcus pyogenes* var. *albus* was made in this work. Agglutination tests were carried out for this purpose. One strain of *N. catarrhalis* was isolated from dog No. 37, and one strain of *M. pyogenes* var. *albus* was isolated from dog No. 7740. *N. catarrhalis* (human strain) No. 101 and *M. pyogenes* var. *albus* (human strain) No. 202 were obtained from the diagnostic laboratory, Michigan Department of Health, Lansing, Michigan. *N. catarrhalis* Abbott No.5 was obtained from Miss Lisa Neu, Department of Bacteriology and Public Health, Michigan State College. All these human strains were newly isolated from the throats of different individuals.

Preparation of antigens:

The antigens were prepared from the above cultures grown on tryptose agar slants at 37°C for 24 hours, and washed off with 0.5% phenolated saline. The heavy suspension was filtered through a cotton filter into bottles with glass beads, shaken and diluted to turbidity No.1 of McFarland's nephelometer. This turbidity equalled approximately 300,000,000 organisms per ml.

Preparation of antisera:

Each strain of the above organisms was injected into chickens and rabbits for the production of antibodies. These animals were injected intravenously with gradually increased amounts of 24-

hour living cultures (0.25 ml., 0.5 ml., 0.75 ml., and 1 ml.) as shown in tables 16 and 20. Killed cultures were also used for the production of antiserum of M. pyogenes var. albus as shown in table 21. These cultures were heated at 65° to 70°C for one hour in the water bath. Injections were made twice a week for a period of three to four weeks. The animals were bled six to ten days after the last injection.

Agglutination tests:

For the cross agglutination reaction between the different strains of N. catarrhalis, antisera from chickens produced by injection with each organism were used. Four dilutions 1-25, 1-50, 1-100, and 1-200, of the antisera were introduced into separate tubes. To the fifth tube negative serum from a normal chicken was added. There was no serum in the sixth tube. The last two tubes were used as controls. One ml. of the antigen was added to each of these tubes. After mixing the contents thoroughly, these tubes were incubated at 37°C. Results were read after 24 and 48 hours of incubation.

Agglutination tests were also set up in the same manner with antiserum No. 37 from rabbits, using antigen of the three strains of N. catarrhalis. Normal rabbits serum was used as control.

In the same manner cross agglutination tests on M. pyogenes var. albus from dogs and man were made. Antisera from rabbits produced by injection of living and killed cultures were used. Normal rabbit serum was employed as control.

RESULTS

I. Comparison of Bacterial Florae in Distemper Infected Dogs and Noninfected Dogs

Culture from the eyes and nose of 22 dogs with diagnoses other than distemper were used for comparison with those of 14 dogs infected with distemper during the same season. The organisms found in each case are recorded in tables 1 and 2, and the comparison are shown in table 3.

TABLE 1
ORGANISMS FOUND IN OCULAR AND NASAL DISCHARGES
OF 22 DOGS NOT AFFECTED WITH DISTEMPER

Case No.	Age	Sex	Clinical diagnosis	Amount of growth	Organisms found
5686	*	M	Foreign body intestine	x	M. pyogenes var. albus M. candidus M. epidermidis
7037	14 mo.	F	Mammary neoplasm	x	M. citreus
7131	2 yr.	M	Castration	x	M. pyogenes var. albus M. candidus Hemolytic strep. group C
7193	15 mo.	M	Radial paralysis	x	Hemolytic strep. group C

* Owner unable to give correct age

TABLE 1 (Continued)

Case No.	Age	Sex	Clinical diagnosis	Amount of growth	Organisms found
7421	1 yr.	M	Fractured humerus	xx	M. pyogenes var. albus M. pyogenes var. aureus M. varians M. epidermidis Hemolytic strep. group D
7454	10 wk.	F	Ear trim	x	M. pyogenes var. albus Hemolytic strep. group C
7455	10 wk.	M	Ear trim	xx	M. pyogenes var. aureus M. sp. Hemolytic strep. group C N. catarrhalis
7469	6 yr.	M	Helminthiasis	xx	M. pyogenes var. albus M. epidermidis M. flavus Hemolytic strep. group C group D
7524	5 yr.	M	Fractured humerus	xx	M. epidermidis M. candidus Hemolytic strep. N. catarrhalis
7565	8 mo.	F	Ear trim	xx	M. pyogenes var. albus Nonhemolytic strep. Ps. aeruginosa
7579	*	F	Helminthiasis	x	M. pyogenes var. albus
7640	4 mo.	F	Chorea (eye with discharge)	xx	M. pyogenes var. albus M. candidus M. aurantiacus Hemolytic strep. Nonhemolytic strep. Shigella sp.

* Owner unable to give correct age

TABLE 1 (Continued)

Case No.	Age	Sex	Clinical diagnosis	Amount of growth	Organisms found
7645	3 yr.	F	Ovariectomy	x	M. pyogenes var. albus
7685	3 yr.	M	Eye with serous discharge	xx	M. flavus Nonhemolytic strep. A. aerogenes N. catarrhalis
7688	2 yr.	F	Helminthiasis	x	M. pyogenes var. albus M. epidermidis
7703	4 yr.	M	Eczema	x	M. pyogenes var. albus
7708	*	M	Fractured tibia	x	M. aurantiacus Hemolytic strep. group C N. catarrhalis
7726	1 yr.	M	Castration	x	M. epidermidis M. flavus Hemolytic strep. group C
7734	8 yr.	F	Dermatitis	xx	M. epidermidis M. pyogenes var. albus Hemolytic strep. group C Viridans strep.
7744	9 yr.	F	Dermatitis	xx	M. epidermidis Hemolytic strep. group C N. catarrhalis
1	1 yr.	M	Normal dog	x	M. candidus
1a	2 mo.	F	Ear trim	x	M. pyogenes var. albus M. aurantiacus

Note: Amount of growth in the area of inoculation on blood agar plates indicated by:
xxxx Numerous colonies; xx Less than 100 colonies
xxx Less than 500 colonies; x 1-20 colonies

* Owner unable to give correct age

TABLE 2
ORGANISMS FOUND IN OCULAR AND NASAL DISCHARGES
OF 14 UNTREATED DOGS AFFECTED WITH DISTEMPER

Case No.	Age	Sex	Clinical diagnosis	Amount of growth	Organisms found
7740	1 yr.	F	Distemper with convulsion	xx	Hemolytic strep. group C N. catarrhalis
7690	7 yr.	F	Distemper and pneumonia with labored breathing	xxx	M. pyogenes var. albus M. aurantiacus Br. bronchiseptica
7618	4 yr.	F	Distemper with severe convulsion	xx	M. epidermidis
1	Small	F	Distemper	xx	M. pyogenes var. albus Br. bronchiseptica
2	Small	F	Distemper	xxx	M. pyogenes var. albus Nonhemolytic strep. Br. bronchiseptica N. catarrhalis
3	*	F	Distemper	xx	M. pyogenes var. albus Hemolytic strep. group C N. catarrhalis
4	*	M	Distemper	x	Hemolytic strep. group C
5	*	M	Distemper	xxx	M. pyogenes var. albus Hemolytic strep. group D E. coli N. catarrhalis

* Age unknown
These dogs were obtained from The Humane Society

TABLE 2 (Continued)

Case No.	Age	Sex	Clinical diagnosis	Amount of growth	Organisms found
6	*	M	Distemper	xxx	M. pyogenes var. albus M. sp. E. coli A. aerogenes
7	3 yr.	F	Distemper	xx	M. pyogenes var. albus
8	*	F	Distemper	xx	M. pyogenes var. albus M. pyogenes var. aureus Nonhemolytic strep.
9	*	F	Distemper	xxxx	M. epidermidis Hemolytic strep. group C A. aerogenes Pr. mirabilis Br. bronchiseptica
10	*	M	Distemper	x	Viridans strep.
24	*	M	Distemper	xxx	M. pyogenes var. albus Hemolytic strep. group C Pr. mirabilis

Note: Amount of growth in the area of inoculation on blood agar plates indicated by:
xxxx Numerous colonies
xxx Less than 500 colonies
xx Less than 100 colonies
x 1-20 colonies

* Age unknown

These dogs were obtained from The Humane Society

TABLE 3
COMPARISON OF BACTERIAL FLORAE OF DISTEMPER INFECTED AND NONINFECTED DOGS

Name of organisms	Percentage of dogs found with the organisms	
	14 infected dogs	22 noninfected dogs
A. Micrococci		
M. pyogenes var. albus	64.2	59.0
M. pyogenes var. aureus	7.1	9.1
M. aurantiacus	7.1	13.1
M. candidus	-	22.7
M. citreus	-	4.5
M. epidermidis	14.3	36.3
M. flavus	-	13.6
M. varians	-	4.5
M. sp.	7.1	9.1
B. Streptococci		
Hemolytic strep.		
group A	-	-
group B	-	-
group C	35.5	40.8
group D	7.1	13.6
Viridans strep.	7.1	27.2
Nonhemolytic strep.	14.3	13.6
C. Gram-negative bacilli		
Br. bronchiseptica	28.5	-
E. coli and A. aerogenes	14.3	4.5
Ps. aeruginosa	-	4.5
Pr. mirabilis	14.3	-
Shigella sp.	-	4.5
D. Gram-negative cocci		
N. catarrhalis	28.5	22.7

Note: Identification of the organisms was based on biochemical reactions according to Bergey's Manual of Determinative Bacteriology (1948)

II. Comparison of Bacterial Florae Found in Distemper Infected Dogs During Different Seasons

According to the clinical records of the veterinary hospital at Michigan State College, 713 dogs were found to have canine distemper during the period of one year from June 1948 to May 1949. The number of cases reported as canine distemper in each month is indicated in table 4. The seasonal incidence and enzootic period of distemper during the winter season, from November to April, is shown in figure 1. The various organisms found in terms of percentage of cases during this period are shown in table 5.

TABLE 4
NUMBER OF CASES OF CANINE DISTEMPER
IN VARIOUS MONTHS

Month	Year	Number of cases per month	Number of cases per day
June	1948	46	1.53
July	1948	24	.77
August	1948	24	.77
September	1948	16	.53
October	1948	25	.81
November	1948	64	2.13
December	1948	84	2.71
January	1949	114	3.68
February	1949	81	2.89
March	1949	99	3.19
April	1949	81	2.70
May	1949	55	1.77

TABLE 5
VARIOUS ORGANISMS FOUND IN DOGS INFECTED WITH DISTEMPER DURING DIFFERENT SEASONS

Name of organisms	Percentage of dogs harboring the various organisms			
	Summer (June-Aug.) 1948	Fall (Sept.-Dec.) 1948	Winter (Jan.-Mar.) 1949	Spring (Mar.-May) 1949
A. Micrococci				
M. pyogenes var. albus	73.3	44.4	100.0	62.5
M. pyogenes var. aureus	3.3	11.1	-	-
M. aurantiacus	10.0	22.2	54.5	12.5
M. candidus	10.0	11.1	36.3	12.5
M. conglomeratus	-	-	-	12.5
M. caseolyticus	3.3	66.6	9.1	12.5
M. epidermidis	30.2	22.2	27.2	12.5
M. flavus	6.6	22.2	9.1	-
M. freudenreichii	-	11.1	-	-
M. luteus	3.3	11.1	-	12.5
M. varians	3.3	33.3	18.1	-
B. Streptococci				
Hemolytic strep. group A	-	-	-	-
group B	3.3	-	-	-
group C	36.6	22.2	54.6	-
group D	36.6	22.2	91.0	-
Viridans strep.	13.3	55.5	18.1	62.5
Nonhemolytic strep.	30.0	66.6	27.2	25.0
C. Gram-negative bacilli				
Br. bronchiseptica	16.6	33.3	54.5	50.0
E. coli and A. aerogenes	20.0	33.3	36.3	12.5
Ps. aeruginosa	3.3	-	-	-
Pr. mirabilis	6.6	11.1	-	12.5
Shigella sp.	3.3	11.1	-	-
D. Gram-negative cocci				
N. catarrhalis	33.3	-	9.1	-

Note: Identification of the organisms was based on biochemical reactions according to Bergey's Manual of Determinative Bacteriology (1948)

III. Bacteriological Studies on the Effects of Treatment

A study of the effects of local treatment with various therapeutic agents in canine distemper was made on 46 cases. The amount of growth on blood agar plates made before and after treatment, is indicated by a number of Xs on an arbitrary scale. In all, swabs were examined as follows:

1. Distemper serum

Distemper serum was dropped into the eyes and nose of four dogs infected with distemper. The organisms found before and after the treatment are shown in table 6 and the ineffectiveness of local serum therapy is indicated in figure 10.

2. Sulfathiazole ointment (five percent)

This drug was used on seven dogs which showed some degree of clinical improvement. The results obtained from this agent applied locally is shown in table 7 and figure 10.

3. Sodium sulfacetamide solution (30 percent)

This solution was used as eye and nose drops on four dogs affected with distemper. Not very much evidence of improvement was shown clinically. The results are indicated in table 8 and figure 10.

4. Baciguent ointment (500 units of bacitracin per gram)

The drug was applied to the conjunctivae once daily for three days. No change was apparent during the first 48 hours but there was a sudden drop in growth after the third application in case No. 5708. The results are shown in table 9 and figure 10.

5. Bacitracin ointment (500 units per gram)

Six dogs were treated with this ointment. There was some evidence of gradually decreasing growth. The activity of this drug is shown in table 10 and figure 10.

6. Streptomycin solution (100,000 S units per ml.)

This solution was dropped into the nose and eyes of four dogs infected with distemper. Clinical observations showed very slight improvement after treatment and this was not constant. The antibacterial activity of this agent is indicated in table 11 and figure 10.

7. Potassium penicillin ointment (28,600 units per gram)

This compound was used on five dogs. The results are shown in table 12 and figure 10. Not very much effectiveness can be seen in these five cases. Unfortunately one animal was infected with *Proteus* which was insensitive to penicillin treatment.

8. Calcium penicillin ointment (14,300 units per gram)

This ointment was found to be the most effective agent. It was applied locally in the eyes and nose of 13 dogs. Definite antibacterial activity was shown at the end of four hours after treatment as indicated in figure 10. Eight of the dogs showed beneficial results clinically, and five of them gave sterile swabs 72 hours after the first application. The results are shown in table 13.

TABLE 6

BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF FOUR DOGS FOLLOWING TREATMENT WITH DISTEMPER SERUM

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
1974	xxxx M. caseolyticus M. varians E. coli Nonhemolytic strep.	xxxx M. caseolyticus Viridans strep.	xxxx M. caseolyticus M. pyogenes var. albus E. coli Viridans strep.	xxx M. caseolyticus M. epidermidis Viridans strep.	xxx M. caseolyticus M. aurantiacus Nonhemolytic strep.
2048	xx M. luteus M. caseolyticus Hemolytic strep. group C A. aerogenes	xxx M. flavus M. caseolyticus Hemolytic strep. group C	xxx M. freudenreichii M. caseolyticus Hemolytic strep. group C		

TABLE 6 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
2057	x Br. bronchi- septica	x Br. bronchi- septica Hemolytic strep. group C	x Br. bronchi- septica	x Br. bronchi- septica	
2074	x M. varians Shigella sp.	None	x M. caseolyti- cus	xxx M. caseolyticus A. aerogenes	

Note: xxxx Numerous colonies
xxx Less than 500 colonies
xx Less than 100 colonies
x 1-20 colonies
None no growth

TABLE 7

BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF SEVEN DOGS FOLLOWING TREATMENT WITH SULFATHIAZOLE OINTMENT

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		4 hours	24 hours	48 hours	72 hours
11	xxx M. pyogenes var. albus Nonhemolytic strep. Shigella sp.		xxx M. pyogenes var. albus Nonhemolytic strep.		
12	xx M. epidermidis		xx M. epidermidis		
13	xxx M. pyogenes var. albus M. sp.		xx M. sp.		
14	xx M. pyogenes var. albus		None		

TABLE 7 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		4 hours	24 hours	48 hours	72 hours
15	xx M. pyogenes var. albus		xx M. pyogenes var. albus		
28	xxx M. aurantiacus M. luteus Hemolytic strep. group C	xxx M. aurantiacus Hemolytic strep. group C Nonhemolytic strep.	x M. aurantiacus Hemolytic strep. group C		xxx M. aurantiacus M. flavus Hemolytic strep. group C
30	x M. pyogenes var. albus Hemolytic strep.	x M. pyogenes var. albus	x M. pyogenes var. albus Hemolytic strep. group C		x M. epidermidis

Note: xxxx Numerous colonies
xxx Less than 500 colonies
xx Less than 100 colonies
x 1-20 colonies
None no growth

TABLE 8
BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF FOUR DOGS FOLLOWING TREATMENT WITH SODIUM SULFACETAMIDE SOLUTION

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
6000	xxxx M. aurantiacus	xx M. aurantiacus M. candidus	x M. aurantiacus M. epidermidis	xxxx M. aurantiacus	
6001*	x M. aurantiacus Hemolytic strep. A. aerogenes		xx M. aurantiacus Hemolytic strep. A. aerogenes	xx M. aurantiacus	x M. aurantiacus Viridans strep.
7715	x M. aurantiacus M. pyogenes var. albus		x M. aurantiacus	xx M. aurantiacus	x M. aurantiacus
112	xxxx M. pyogenes var. albus Hemolytic strep. group C	xxxx M. pyogenes var. albus Hemolytic strep. group C	xxxx M. pyogenes var. albus Hemolytic strep. group C	xxxx M. pyogenes var. albus	

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1-20 colonies None No growth

* On the ninth day (the fifth day after the last swab) the growth was xxxx (M. aurantiacus and E. coli)

This work was done in summer 1950.

TABLE 9
BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF
THREE DOGS FOLLOWING TREATMENT WITH BACIGUENT OINTMENT

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
5672	xxxx M. pyogenes var. albus Br. bronchi-septica	xxxx M. pyogenes var. albus Br. bronchi-septica	xxxx M. pyogenes var. albus Br. bronchi-septica		
5679	xxxx M. pyogenes var. albus Hemolytic strep. group D Hemophilis sp. Br. bronchi-septica	xxxx M. pyogenes var. albus Hemolytic strep. group D			xxxx M. pyogenes var. albus Hemolytic strep.
5708	xxxx M. pyogenes var. albus Hemolytic strep. E. coli	xxxx M. pyogenes var. albus	xxxx M. pyogenes var. albus	x Hemolytic strep. group D	

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1–20 colonies None no growth

TABLE 10
BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF SIX DOGS FOLLOWING TREATMENT WITH BACITRACIN OINTMENT

Case No.	Organisms Found before treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
4759	xxxx M. pyogenes var. albus	xxx M. pyogenes var. albus	x M. pyogenes var. albus	None	None
4930	xxxx M. pyogenes var. albus	xxx M. pyogenes var. albus	Dog died of convulsion		
4891*	xxxx M. candidus M. caseolyticus Br. bronchiseptica	xxxx M. pyogenes var. albus Nonhemolytic strep. Br. bronchiseptica	xxx M. pyogenes var. albus Br. bronchiseptica	xx M. pyogenes var. albus Viridans strep. Hemophilis sp.	xxx M. pyogenes var. albus M. caseolyticus Nonhemolytic strep.
4966**	x M. pyogenes var. albus M. aurantiacus Hemolytic strep.	xx M. pyogenes var. albus M. aurantiacus Hemolytic strep. E. coli			xxxx M. pyogenes albus M. aurantiacus Hemolytic strep. group C

TABLE 10 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
5062***	x M. pyogenes var. albus M. aurantiacus Nonhemolytic strep. group D	x M. pyogenes var. albus M. aurantiacus Nonhemolytic strep. group D N. catarrhalis			xxxx M. epidermidis M. candidus Viridans strep.
5122****	xxxx M. pyogenes var. albus Nonhemolytic strep.	x M. candidus M. epidermidis	xx M. pyogenes var. albus M. candidus M. epidermidis Nonhemolytic strep. Br. bronchiseptica		

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1-20 colonies None no growth

* This dog was treated continuously with the drug for 17 days. The cultures were xxxx on the 21st and 23rd day (_M. pyogenes_ var. _albus_ and _Br. bronchiseptica_).

** This dog was treated continuously with this drug for 18 days. The cultures were xxx until the 18th day (_M. pyogenes_ var. _albus_, _M. epidermidis_ and _Br. bronchiseptica_)

*** After the 16 days of treatment with this drug this dog was treated with calcium penicillin ointment and the dog recovered.

**** This dog was treated continuously with this drug for 11 days. Cultures were xx on the 5th, 6th and 7th day; xxx on the 8th day; xxxx on the 11th day. The organisms were the same as those found in the 24 hours culture.

TABLE 11
BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF FOUR DOGS FOLLOWING TREATMENT WITH STREPTOMYCIN SOLUTION

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
2193	x M. candidus Viridans strep.	x M. candidus Viridans strep.	x Nonhemolytic strep. Viridans strep.		
2247	xxx M. pyogenes var. albus Hemolytic strep. group C Br. bronchi-septica	xx M. epidermidis Viridans strep. Nonhemolytic strep. Br. bronchi-septica	x M. pyogenes var. albus	x Nonhemolytic strep. Br. bronchiseptica	
2434	x M. pyogenes var. albus M. caseolyticus M. varians Viridans strep.	x Nonhemolytic strep. Viridans strep.			
3006	xx M. aurantiacus	xx Nonhemolytic strep.	x M. pyogenes var. albus Nonhemolytic strep.		

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1–20 colonies None no growth

TABLE 12
BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF FIVE DOGS FOLLOWING TREATMENT WITH POTASSIUM PENICILLIN OINTMENT

Case No.	Organisms Found before Treatment	Organisms Found after Treatment			
		24 hours	48 hours	72 hours	96 hours
6268*	x M. epidermidis Viridans strep.	xx M. aurantiacus Viridans strep. Br. bronchi-septica	xx Viridans strep. Br. bronchi-septica (streptomycin was applied after this swab)	None	x M. pyogenes var. albus Viridans strep.
6380	xxxx M. pyogenes var. albus M. auarntiacus Viridans strep.	xxxx M. pyogenes var. albus M. aurantiacus Hemolytic strep. group D	xxxx M. pyogenes var. albus M. aurantiacus Viridans strep. E. coli		
6458	x M. pyogenes var. albus	x M. candidus	None		
6517	None	None	None	x Viridans strep.	
6535	xxxx Pr. mirabilis			xxxx Pr. mirabilis	

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1-20 colonies None no growth

* This dog was treated with streptomycin solution after 48 hours of penicillin treatment.

Br. bronchiseptica was obtained on the 5th day culture.

TABLE 13

BACTERIAL FLORAE OF CONJUNCTIVAL AND NASAL DISCHARGES OF THIRTEEN DOGS FOLLOWING TREATMENT WITH CALCIUM PENICILLIN OINTMENT

Case No.	Organisms Found before Treatment	Organisms Found after Treatment				
		4 hours	24 hours	48 hours	72 hours	144 hours
16	xxxx M. pyogenes var. albus Hemol. strep. group C		xx M. pyogenes var. albus			
17	xxxx M. epidermidis Hemol. strep. group C Nonhemol. strep.		x M. epidermidis			xxxx A. aerogenes
18	x M. pyogenes var. albus Nonhemol. strep. group D		None			xxxx M. pyogenes var. albus Nonhemol. strep.
6895	xx Nonhemol. strep. Br. bronchi-septica				x Viridans strep.	

TABLE 13 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment: 4 hours	24 hours	48 hours	72 hours	144 hours
32	xxxx M. pyogenes var. albus M. flavus Nonhemol. strep. Hemol. strep. group D	x M. pyogenes var. albus	None	None	None	
33	xx M. pyogenes var. albus M. aurantiacus M. flavus Ps. aeruginosa	x M. pyogenes var. albus Hemol. strep. group B group D	x Hemol. strep. group D N. catarrhalis	None	None	
34	x M. epidermidis M. varians Hemol. strep. group D	x M. pyogenes var. albus Hemol. strep. group D Br. bronchi-septica	x Br. bronchi-septica		Dog destroyed	
35	x M. caseolyticus Nonhemol. strep.	x M. sp. Nonhemol. strep. N. catarrhalis	xxx N. catarrhalis Br. bronchi-septica		xx M. pyogenes var. albus Nonhemol. strep.	

TABLE 13 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment				
		4 hours	24 hours	48 hours	72 hours	144 hours
36	x M. pyogenes var. albus M. candidus M. epidermidis Nonhemol. strep. N. catarrhalis	x M. pyogenes var. albus Nonhemol. strep.	x M. pyogenes var. albus Nonhemol. strep.	None	None	
37	xxxx M. pyogenes var. albus M. candidus Nonhemol. strep.	xx M. pyogenes var. albus N. catarrhalis	x M. pyogenes var. albus N. catarrhalis		x M. pyogenes var. albus Nonhemol. strep.	
4432	xxxx Gram-positive rods (hemol.)		x M. pyogenes var. albus M. aurantiacus Nonhemol. strep.	None	None	
6847*	xx M. caseolyticus Viridans strep.			x M. pyogenes var. albus Nonhemol. strep.		x Viridans strep. Br. bronchi-septica

TABLE 13 (Continued)

Case No.	Organisms Found before Treatment	Organisms Found after Treatment				
		4 hours	24 hours	48 hours	72 hours	144 hours
7282	xx M. pyogenes var. albus Nonhemol. strep. Br. bronchiseptica		x M. epidermidis Nonhemol. strep.	x M. pyogenes var. albus Br. bronchiseptica		

Note: xxxx Numerous colonies xxx Less than 500 colonies xx Less than 100 colonies
x 1–20 colonies None no growth

* This dog was treated with this ointment daily for six days. Cultures were x on the 7th, 8th, 10th and 11th day. The organisms were nonhemolytic streptococci and <u>Br.</u> <u>bronchiseptica.</u>

Data on case Nos. 16, 17, 18, 32, 33, 34, 35, 36, 37, and 4432 published in Cornell Veterinarian, 40: 4–10, 1950. (Hsiung et al.)

TABLE 14
COMPARISON OF AVERAGE AMOUNT OF BACTERIAL GROWTH IN CULTURES FROM CONJUNCTIVAL AND NASAL DISCHARGES FOLLOWING TREATMENT WITH VARIOUS THERAPEUTIC AGENTS

Name of therapeutic agents used	No. of cases treated	Growth before treatment Xs	Growth after treatment Xs					
			4 hours	24 hours	48 hours	72 hours	96 hours	144 hours
Distemper serum	4	2.0		2.0	2.3	2.3	3.0	
Sulfathiazole	7	2.3	2.0	1.6		2.0		
Na sulfacetamide	4	2.5		3.0	2.5	3.0	1.0	
Baciguent ointment	3	4.0		4.0	4.0	1.0	4.0	
Bacitracin ointment	6	3.1		2.8	2.0	2.0	2.5	
Streptomycin solution	4	1.8		1.5	1.0	1.0		
K penicillin ointment	5	2.0		2.5	1.5	2.5	1.0	
Ca penicillin ointment	13	2.4	1.2	1.1	0.3	0.6		3.0

Note: Number of Xs indicates the amount of growth according to arbitrary scale

Legend see note on page 43.

IV. SEROLOGICAL STUDIES ON NEISSERIA CATARRHALIS AND MICROCOCCUS PYOGENES VAR. ALBUS ISOLATED FROM THE DOGS AFFECTED WITH DISTEMPER

1. *Neisseria catarrhalis*

The biochemical reactions of the two human strains (cultures No. 101 and Abbott No. 5) and one strain from dog No. 37 of *N. catarrhalis* are shown in table 15. The period of immunization of the animals for the production of antisera and the time of bleeding are shown in table 16. Tables 17 and 18 indicate the results of the cross agglutination reactions of antisera from chickens and rabbits respectively. Although there is definite cross agglutination between strains No. 101 and Abbott No. 5, there is no evidence that the two human strains of *N. catarrhalis* and the strain isolated from dog No. 37 are antigenically related. Table 17 indicates that the antisera from the chickens give stronger positive results than the antisera from rabbits (table 18) used in these agglutination tests.

2. *Micrococcus pyogenes* var. *albus*

The biochemical reactions of the human strain (culture No. 202) and strain No. 7740, isolated from a dog, are shown in table 19. Only rabbits were used for the production of the antisera for these two organisms. Table 20 and 21 show the period of immunization with living and killed organisms and time of bleeding of the animals. The results of agglutination are shown in table 22. Here again it is indicated that there is not much serological relationship between these two strains of *M. pyogenes* var. *albus*.

TABLE 15
BIOCHEMICAL REACTIONS OF THE THREE STRAINS OF NEISSERIA CATARRHALIS

Media	Culture number		
	101 (human origin)	Abbott 5 (human origin)	37 (canine origin)
Oxidase test	+	+	+
Dextrose	-	-	-
Lactose	-	-	-
Maltose	-	-	-
Mannitol	-	-	-
Sucrose	-	-	-
Indol	-	-	-
Litmus	-	-	-
Nitrate	+	-	+

Note: + Positive reaction
- Negative reaction

These three strains are all gram-negative diplococci. They are nonhemolytic with big colonies on blood agar plates. On first isolation from dogs, they showed abundant growth on tryptose agar slants. The human strain showed only scanty growth on primary isolation.

TABLE 16
IMMUNIZATION OF ANIMALS WITH NEISSERIA CATARRHALIS (LIVING CULTURE)

Antigen source	101 (human)		Abbott 5 (human)			37 (canine)					
Animal used	Chicken		Chicken			Chicken			Rabbit		
	A	D	B	E	F	C	G	H	A	B	C
Date of injection in ml.											
11-24-49	.25		.25			.25			.25		
11-30-49	.5		.5			.5			.5		
12-4-49	.75		.75			.75			.75		
12-9-49	1		1			1			1		
12-14-49	1	.25	1	.25	.25	1	.25	.25	1		
12-16-49	1	.5	1	.5	.5	1	.5	.5	1		
12-20-49	1	.75	1	.75	.75	1	.75	.75	1		
12-30-49	1	1	1	1	1	1	1	1	1		
1-3-50	1	1	1	1	1	1	1	1	1		
1-6-50	1	1	1	1	1	1	1	1	1		
1-10-50	1	1	1	1	1	1	1	1	1		
1-13-50		1		1	1		1	1			
1-19-50 Bled											

TABLE 16 (Continued)

Antigen source	101 (human)		Abbott 5 (human)			37 (canine)					
Animal used	Chicken		Chicken			Chicken			Rabbit		
	A	D	B	E	F	C	G	H	A	B	C
Date of injection in ml.											
1-25-50 Bled after 48 hours starvation											
2-2-50		1	1		1	1				.25	.25
2-6-50		1	1		1	1				.5	.5
2-10-50		1	1		1	1				.75	.75
2-13-50		1	1		1	1				1	1
2-17-50		1	1		1	1				1	1
2-23-50		Bled	Bled		Bled	Bled				1	1
2-27-50										1	1
3-1-50										1	1
3-3-50										1	1
3-9-50										Bled	Bled

TABLE 17
CROSS AGGLUTINATION REACTION BETWEEN THE THREE STRAINS OF NEISSERIA CATARRHALIS WITH CHICKEN SERA

Antigen source	101 (human)						Abbott 5 (human)						37 (canine)					
Serum dilution	1-25	1-50	1-100	1-200	N	0	1-25	1-50	1-100	1-200	N	0	1-25	1-50	1-100	1-200	N	0
Serum No. (animal No.)																		
Antiserum A	+++	++	+	-	-	-	+++	++	+	-	-	-	-	-	-	-	-	-
Antiserum D	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antiserum B	cl.	++	++	+	-	-	cl.	cl.	++	++	-	-	cl.	cl.	cl.	cl.	-	-
Antiserum E	+++	++	+	+	-	-	+++	++	+	-	-	-	-	-	-	-	-	-
Antiserum F	+	+	+	±	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Antiserum C	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	++	+	-	-
Antiserum G	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	+	+	-	-
Antiserum H	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	+	-	-	-

Note:
- +++ Strong positive reaction
- ++ Marked positive reaction
- + Slight positive reaction
- ± Doubtful
- - Negative reaction

- cl. Cloudy
- N Normal chicken serum
- 0 No serum

Antisera A and D were produced from antigen strain 101
Antisera B, E and F were produced from antigen Abbott 5
Antisera C, G and H were produced from antigen strain 37

TABLE 18

AGGLUTINATION REACTION OF THE THREE STRAINS OF NEISSERIA CATARRHALIS WITH ANTISERUM 37 FROM RABBITS

Antigen source	101 (human)						Abbott 5 (human)						37 (canine)					
Serum dilution	1-25	1-50	1-100	1-200	N	0	1-25	1-50	1-100	1-200	N	0	1-25	1-50	1-100	1-200	N	0
Serum No. (animal No.)																		
Antiserum 37 rabbit A	-	-	-	-	-	-	-	-	-	-	-	-	+	±	-	-	-	-
Antiserum 37 rabbit B	-	-	-	-	-	-	-	-	-	-	-	-	++	++	-	-	-	-
Antiserum 37 rabbit C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-

Note: +++ Strong positive reaction
++ Marked positive reaction
\+ Slight positive reaction
± Doubtful
\- Negative reaction

N Normal rabbit serum
0 No serum

TABLE 19
BIOCHEMICAL REACTIONS OF THE TWO STRAINS
OF MICROCOCCUS PYOGENES VAR. ALBUS

Media	Culture number	
	202 (human origin)	7740 (canine origin)
Ammonium phosphate agar	-	-
Nitrate broth	+	+
Gelatin liquefication	+ (24 hours)	+ (48 hours)
Litmus milk	acid and curd	acid and curd
Indol	-	-
Dextrose	+	+
Lactose	+	+
Maltose	+	+
Mannitol	+ (one week)	+ (one week)
Sucrose	+	+
Glycerol	+	+
Raffinose	+	+

Note: + Positive reaction
- Negative reaction

Both organisms are gram-positive cocci, heavy whitish growth on tryptose agar slants, and alpha hemolytic colonies on blood agar plates.

TABLE 20

IMMUNIZATION OF RABBITS WITH MICROCOCCUS PYOGENES VAR. ALBUS (LIVING CULTURE)

Antigen source	202 (human)		7740 (canine)	
Animal number	I	II	III	IV
Date of injection in ml.				
2-24-50	.25	.25	.25	.25
2-27-50	.25	.25	.25	.25
3-1-50	.5	.5	.5	.5
3-3-50	.75	.75	.75	.75
3-9-50	1	1	1	1
3-11-50	1	1	1	1
3-14-50	1	1	1	1
3-24-50 Bled				

TABLE 21

IMMUNIZATION OF RABBITS WITH MICROCOCCUS PYOGENES VAR. ALBUS (KILLED CULTURE)

Antigen source	202 (human)		7740 (canine)	
Animal number	V	VI	VII	VIII
Date of injection in ml.				
2-2-51	.5	.5	.5	.5
2-6-51	1	1	1	1
2-9-51	1	1	1	1
2-13-51	1	1	1	1
2-16-51	1	1	1	1
2-20-51	1	1	1	1
2-23-51	1	1	1	1
2-27-51	1	1	1	1
3-2-51	1	1	1	1
3-17-51 Bled				

TABLE 22
CROSS AGGLUTINATION REACTION BETWEEN TWO STRAINS OF MICROCOCCUS PYOGENES VAR. ALBUS

Antigen source	202 (human)					7740 (canine)				
Serum dilution	1-25	1-50	1-100	1-200	N O	1-25	1-50	1-100	1-200	N O
Serum No. (animal No.)										
Antiserum I	+++	++	++	++	- -	±	-	-	-	- -
Antiserum II	+++	++	++	++	- -	±	±	-	-	- -
Antiserum V	+++	+++	++	++	- -	-	-	-	-	- -
Antiserum VI	+++	+++	++	++	- -	-	-	-	-	- -
Antiserum III	±	±	-	-	- -	++	+	+	±	- -
Antiserum IV	±	-	-	-	- -	+	+	+	±	- -
Antiserum VII	-	-	-	-	- -	+	+	+	±	- -
Antiserum VIII	-	-	-	-	- -	++	+	+	+	- -

Note:

+++ Strong positive reaction
++ Marked positive reaction
\+ Slight positive reaction
± Doubtful
\- Negative reaction

N Normal rabbit serum
O No serum

DISCUSSION

The results (tables 1, 2, and 3) show a variation in the different species of micrococci, streptococci, and gram-negative bacilli present in the discharges of the eyes and nose. Br. bronchiseptica, which was considered by a number of investigators as a secondary invader in canine distemper, was isolated from 28.5 percent of the cases of infected dogs. This organism was not found in the noninfected animals during the period of comparison, as shown in table 3. Blood agar plates from the 60 infected dogs made before treatment was started, always showed heavy growth. In contrast, the cultures made from the 22 dogs, not showing clinical symptoms of distemper, usually presented very few colonies. This indicates that the heavy serous or mucopurulent discharge was caused by bacteria. It may be that the virus of Carré lowered the resistance of the animals and thus gave secondary invaders an opportunity to multiply freely.

At the beginning of this work, MacConkey, sodium azide, and blood agar plates were used for the isolation of organisms from the ocular and nasal discharges. Later, results showed that the MacConkey and sodium azide plates were not necessary, as the colonies on the blood agar plates were sufficiently discrete for isolation.

Brain-heart infusion and tryptose broth were used to detect any organisms that were absent on the original blood plates. Streptococci seemed to grow very well in tryptose broth.

Identification and classification of the isolated organisms were based upon biochemical reactions according to Bergey's Manual of Determinative Bacteriology (1948). Serological tests were not used because of the large number of organisms isolated at one time and the lack of specific antiserum for each type of organism.

Although there is a seasonal incidence of canine distemper, nothing indicated a seasonal variation in prevalence of the species of the organisms present. An enzootic period from November to April with the peak in January is definitely shown (figure 1).

Strains of *N. catarrhalis*-like organisms were isolated from several cases during the summer and winter (table 5). This is a gram-negative diplococcus which does not ferment dextrose, lactose, maltose, mannitol and sucrose. This organism has never been mentioned in the literature as having any relationship to the canine distemper complex. One author (Givener, 1949) has reported on eye infections from *N. catarrhalis* in human beings. In this case, the inflammation was successfully controlled by daily pledget applications of penicillin.

M. pyogenes var. *albus* was found to be the dominant organism in the ocular and nasal discharges of all the dogs studied during the various seasons. This organism was obtained from all the animals during the winter, and from 73.3 percent of them during the summer time (table 5). As shown in tables 3 and 5, the principal organisms found, besides this one, were *M. epidermidis*, *M. caseolyticus*, *M. aurantiacus*, hemolytic streptococci groups C and D, nonhemolytic streptococci, and Br. bronchiseptica. These microbes are considered as important secondary invaders present in the nose and eyes of dogs affected with distemper.

After bacteriological examinations had been made of cases of canine distemper, various drugs were applied to the eyes and nose to determine the value of local therapy. The ideal agent for optimal local action would be one which is readily soluble, and, at the same time, possesses a high degree of bacteriostasis. The results of treatment as revealed by this study were classified as follows:

1. Good: after local treatment, the swabs from the eye and nose gave negative cultures. The ocular and nasal discharges disappeared promptly, indicating clinical recovery.
2. Questionable: decrease in the number of colonies, but continuance of positive cultures and the presence of a discharge.
3. No effect: there was no reduction in the prevalence of bacteria and the discharges continued undiminished.

Distemper serum has been used parenterally for several decades as a therapeutic agent in canine distemper. Schlotthauer (1949) stated that anticanine distemper serum was the most effective single agent that he has used for the treatment of dogs affected with distemper. In this study distemper serum was applied locally by dropping it in the nose and eyes. No beneficial effect was obtained either bacteriologically or clinically (table 6).

The use of sulfa drugs to reduce the mortality from canine distemper has been reported by many workers. Richtner (1942) claimed that bacteria disappeared rapidly from the nose following local treatment with sulfathiazole in acute inflammatory conditions. Thygeson and Braley (1943) found that five percent sulfathiazole ointment was effective in the treatment of chronic conjunctivitis caused by staphylococci. From table 7 it will be noticed that five percent sulfathiazole ointment was not very effective when used locally. There was decreased growth from the swabs in case 28, but a number of organisms was recovered at the end of 72 hours. Case 14 yielded a pure culture of M. pyogenes var. albus which responded to this ointment; negative cultures were obtained at the end of the 24-hour period of treatment. The relative ineffectiveness of this agent as shown in this study was disappointing. It is possible that the drug did not penetrate into the conjunctiva at a sufficiently rapid rate to produce an effective concentration at the site of infection. It is also possible that sulfathiazole may not possess adequate chemotherapeutic activity to control these mixed infections.

According to Benedict and Henderson (1947) sodium sulfacetamide (30 percent solution) gave the best results in the average case of acute conjunctivitis associated with purulent or mucopurulent discharges in human beings. From the results presented in this study (table 8), it is evident that sodium sulfacetamide was not as effective in dogs as in humans. There was a decreasing number of organisms in case 6000 at the end of the 24 and 48-hour period of treatment, but there was heavy growth at the end of 72 hours. No definite conclusion could be drawn in cases 6001, 7715, and 112. According to Robson and Scott (1943), in the application of sodium sulfacetamide solution, it is not only important to use a sufficient concentration, but it is essential to maintain an adequate level of the drug at the site of the infection for a reasonable period. Due to this fact, the use of this drug in treatment necessitates application every hour for the first 48 hours. Thus, in this study, single application of this solution was definitely less effective.

Baciguent and bacitracin are antibiotics produced by Bacillus subtilis. Baciguent ointment was applied to the conjunctivae once daily for three successive days. Table 9 shows the results of this treatment. No improvement was obtained in the three cases treated. Culture from case 5708 gave a marked decrease of growth at the end of 72 hours. This may be due to the fact that an unsuitable swab was obtained. Bacitracin was reported by Bellows (1948b) as a beneficial agent in local therapy for superficial eye infections. A solution containing 500 units per ml. applied over 24 hours, resulted in negative cultures for a limited period of time. This compound seems to be as effective as penicillin in the treatment of conjunctival infection, and better when penicillin-resistant organisms are involved. Therefore, the use of bacitracin would be advisable and more economical than penicillin. According to this investigation (table 10), case 4759 was infected with a pure culture

of M. pyogenes var. albus and showed good results after treatment with bacitracin ointment. Case 4930 was also caused by a single infection, but the animal died of convulsions before the treatment was completed. No effect was produced in cases 4966, 4891, and 5062. Case 5062 recovered after treatment with calcium penicillin ointment when the use of bacitracin for 16 days had failed. Continuous treatment for more than ten days in cases 4891, 4966, and 5122 showed no improvement. The ineffectiveness of bacitracin in this experiment was surprising. It is possible that the concentration of this drug was not sufficient for local ophthalmic therapy. According to Scudi et al. (1947), bacitracin exhibits a slow diffusion and excretion rate, thus it does not spread rapidly enough to produce the desired result. Also, the bacterial action of bacitracin is chiefly against gram-positive organisms. Thus it is possible that when there are gram-negative bacteria, it will not be as efficient as some other agent.

According to Bellows and Farmer (1947), streptomycin, applied locally, decreases the amount of secondary infection accompanying vaccinia infection of the cornea. Kellberg (1950) found that the main action of streptomycin is to conquer the very persistent, lowgrade bacillary infections that commonly complicate distemper of dogs. Eads (1951b) reported that clinical recovery from canine distemper was not enhanced by the use of a combination of anticanine distemper serum and streptomycin. When streptomycin was administered without serum, death or no change in the character of the disease was observed in three of the four dogs. In this study, a solution of 100,000 S units of streptomycin per ml. was used as drops in the nose and eyes. In table 11, it is shown that there is no definite effectiveness in the use of this drug. Case 2247 showed some decrease in the growth of bacteria, but no effect was obtained in cases 2193, 2434, and 3006. In case 2247 (table 11) and case 6268 (table 12), Br. bronchiseptica was present before treatment and the organism persisted after 72 hours of application. This indicates that streptomycin is of little, if any, value in the treatment of secondary infections associated with canine distemper. In this work, gram-positive organisms and Br. bronchiseptica did not respond to streptomycin therapy. Secondly, according to the experimental penetration studies by Leopold (1949), streptomycin does not penetrate easily to the normal cornea when applied in drop or ointment form. Lastly, organisms quickly develop resistance to streptomycin. Therefore, it is essential to attack the infection early with an adequate concentration.

Potassium penicillin ointment in a concentration of 28,600 units per gram is relatively well tolerated by the eyes of dogs. Questionable results were obtained in case 6458 (table 12), and there was not much effect shown in cases 6268, 6380, and 6517. Ineffective results were to be expected in case 6535, from which Proteus was isolated. Penicillin is useless against this organism. The unsatisfactory results obtained with potassium penicillin ointment may be due to the ointment base (Eads, 1951c). Therefore, the penetrating power of penicillin is insufficient to produce an effective concentration at the site of infection.

Good results were obtained by the complete elimination of the bacterial florae in the conjunctival and nasal discharges of several cases after the application of calcium penicillin ointment in a concentration of 14,300 units per gram. Serous and mucopurulent discharges cleared promptly soon after the drug was applied.

When calcium penicillin ointment was applied in a single treatment, no bacteria were found after 24 to 72 hours in five dogs (cases 18, 32, 33, 36, 4432) because of the bacteriostatic action of the drug (table 13). In case 16, the bacterial growth was decreased, but not eliminated in 24 hours. Case 17 also showed decreased bacterial growth in 24 hours, but it became abundant 144 hours after treatment. Cultures from case 18 showed heavy growth of _M. pyogenes_ var. _albus_ and nonhemolytic streptococci 144 hours after treatment, although blood agar plates at 24 hours were negative. The absorption and excretion of penicillin are rapid in the animal body, therefore a single application of this ointment is insufficient. Repeated treatment is necessary in local therapy. Case 34 showed no change in the amount of growth from the swabs, even though _M. pyogenes_ var. _albus_ and hemolytic streptococci could not be recovered after 24-hour treatment. This dog was destroyed before further data could be collected. In cases 37, 6895, and 7282, the bacterial count was decreased but a few colonies grew 72 hours after treatment. _N. catarrhalis_ disappeared completely 72 hours after the treatment in cases 35 and 37.

The results suggest that local application of calcium penicillin ointment is effective in the treatment of secondary infections accompanying canine distemper.

In the serological study, tables 17 and 18 indicate that there is an immunological relationship between the two strains of N. catarrhalis of human origin although they differ in their power to reduce nitrate (table 15). There is no antigenical relation between the strains of human and canine origin. The results of the serological and biochemical tests are confirmatory with respect to classification of the organism.

Results recorded in table 17 (antiserum from chickens) and table 18 (antiserum from rabbits) show that chickens were better than rabbits for the production of antineisseria serum.

Antisera C and F (table 17) showed turbidity at the first bleeding due to the fact that the birds had been fed recently. There was no cloudiness when the sera were obtained after feed had been withheld for 48 hours. This is due to the high fat content in the blood (Bryan, Link and Alberts, 1950).

The low titers of antisera D and F (table 17) showed that these two birds were poor antibody producers. A prolonged immunizing period was allowed.

In the production of antimicroccus sera, living and killed organisms were used as antigens (tables 20 and 21). There does not appear to be much difference in the titers of the antisera produced by these two methods. According to the results the two strains of M. pyogenes var. albus possess different antigenic characteristics (table 22).

SUMMARY

A bacteriological study was made of 214 swabs from the eyes and nose of 82 dogs. Of these, 22 showed no evidence of having distemper while 14 were untreated dogs affected with canine distemper, and 46 were affected with canine distemper and were treated with various drugs.

M. pyogenes var. albus was found to be the dominant organism present in the conjunctival and nasal discharges of all the dogs. Except for the presence of Br. bronchiseptica in dogs affected with distemper there was not very much difference between the bacterial florae of distemper free dogs and that of distemper infected dogs.

In addition to the above mentioned organisms, other common bacteria, encountered in the eyes and nose, were M. epidermidis, M. caseolyticus, M. aurantiacus, hemolytic streptococci groups C and D, nonhemolytic streptococci, and N. catarrhalis. These organisms are considered to be important secondary invaders accompanying canine distemper.

The clinical data show that canine distemper is seasonal in its prevalence. The period of high incidence being November to April, with the highest peak occurring in January. Some, but not very significant, variations in the florae were observed in the various seasons.

Eight drugs were used as local therapeutic agents during

this investigation. Anticanine distemper serum was useless as far as local application is concerned.

The local use of two of the sulfonamides was not as effective as the results reported in the literature would indicate. Sulfathiazole ointment (five percent) and sodium sulfacetamide solution (30 percent) were used in this study.

Baciguent ointment (500 units of bacitracin per gram) was ineffective. Bacitracin ointment (500 units per gram) was effective when the infection was due to a single gram-positive organism, but of no avail on gram-negative bacteria.

Streptomycin solution (100,000 S units per ml.) was not effective against any of the secondary invaders in canine distemper.

Potassium penicillin ointment (28,600 units per gram) was not as effective as calcium penicillin ointment (14,300 units per gram).

Calcium penicillin ointment gave the best results in the treatment of secondary infection of the eyes and nose. There was marked decrease in the number of bacterial colonies present on culture plates made from the swabs, taken from ocular and nasal discharges, four hours following the application of this ointment. Repeated applications are necessary for lasting beneficial results.

In the serological study the agglutination reaction was employed. This work revealed no antigenic relationship between _N. catarrhalis_ of human and canine origin. The same was true in the case of human and canine strains of _M. pyogenes_ var. _albus_.

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Figure 1 SEASONAL INCIDENCE OF 713 CASES OF CANINE DISTEMPER

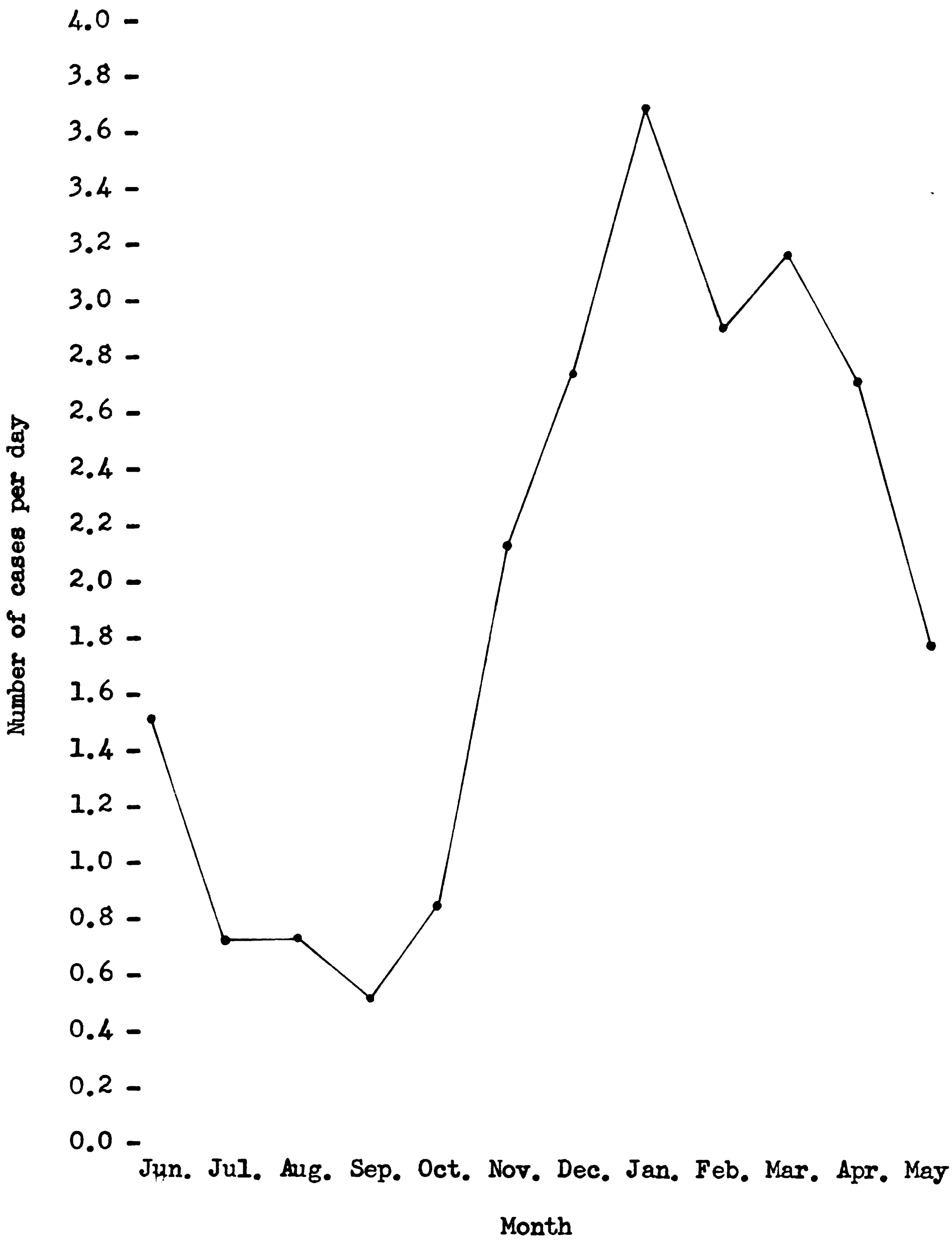
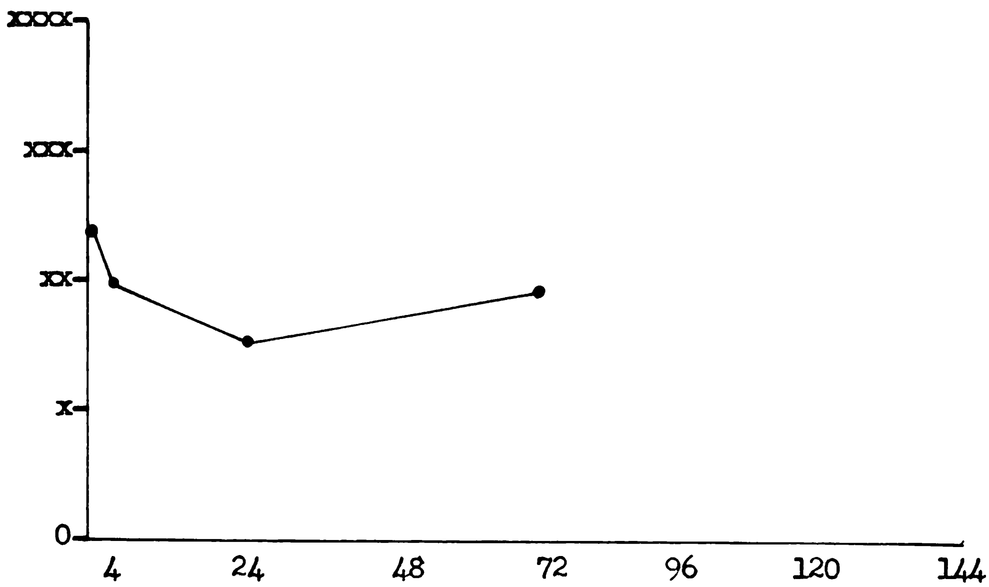


Figure 2 EFFECT OF TREATMENT WITH DISTEMPER SERUM ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES

Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

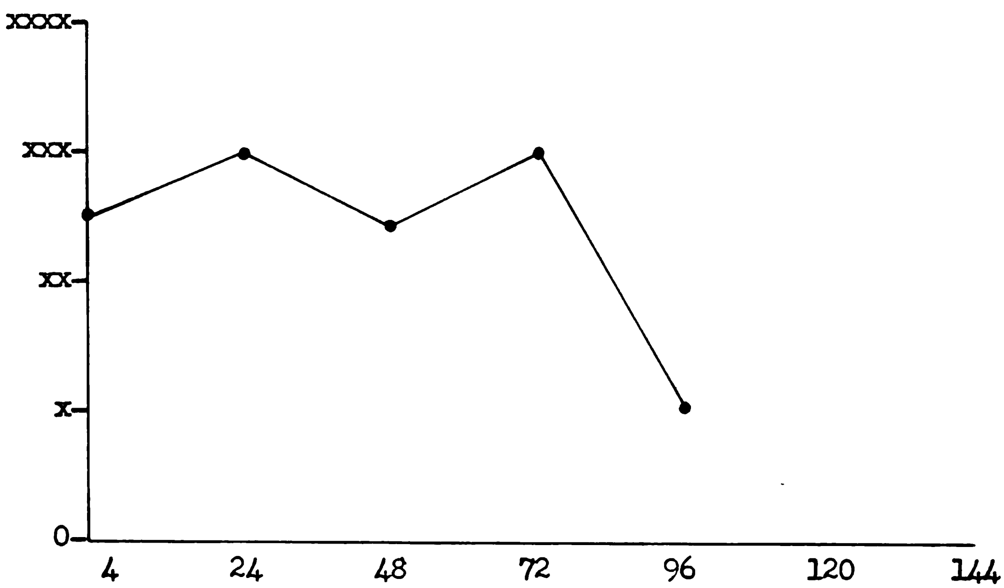
Figure 3 EFFECT OF TREATMENT WITH SULFATHIAZOLE OINTMENT ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Hours after treatment

Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

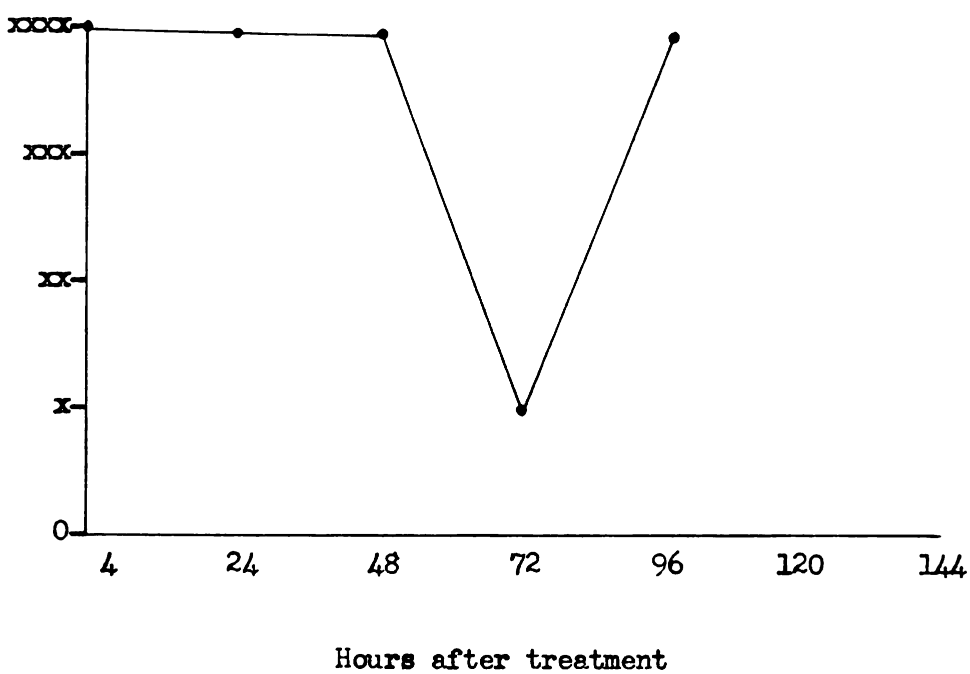
Figure 4 EFFECT OF TREATMENT WITH SODIUM SULFACETAMIDE SOLUTION ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Hours after treatment

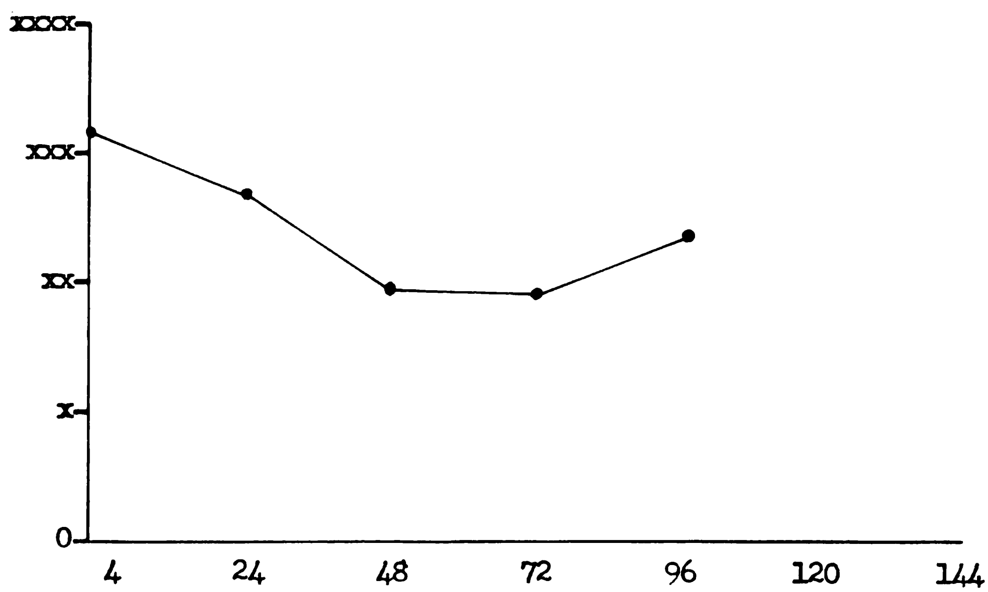
Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

Figure 5 EFFECT OF TREATMENT WITH BACIGUENT OINTMENT ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

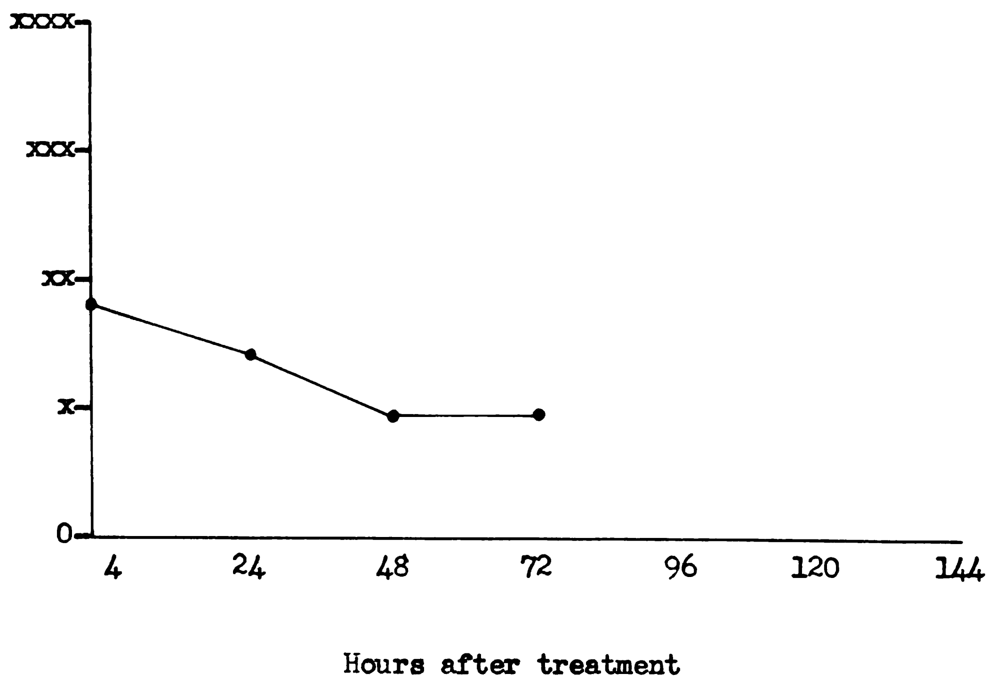
Figure 6 EFFECT OF TREATMENT WITH BACITRACIN OINTMENT ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Hours after treatment

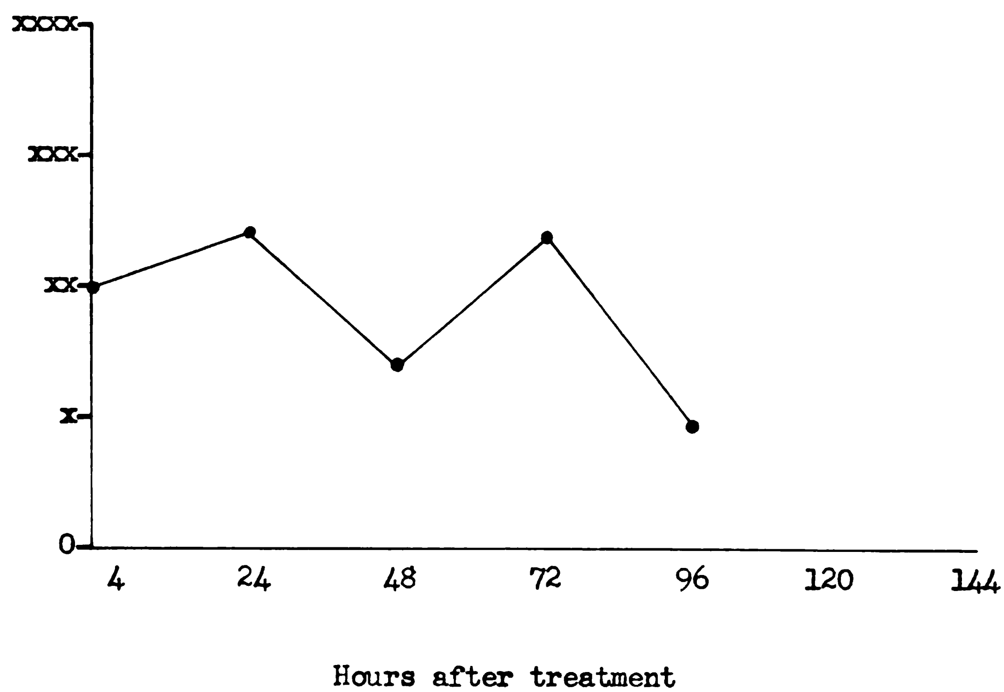
Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

Figure 7 EFFECT OF TREATMENT WITH STREPTOMYCIN SOLUTION ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



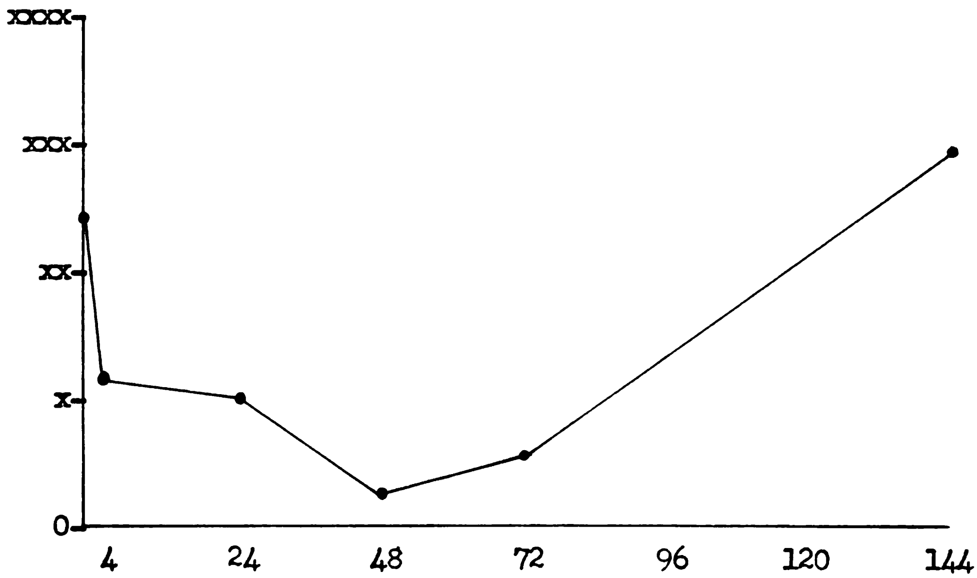
Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

Figure 8 EFFECT OF TREATMENT WITH POTASSIUM PENICILLIN OINTMENT ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

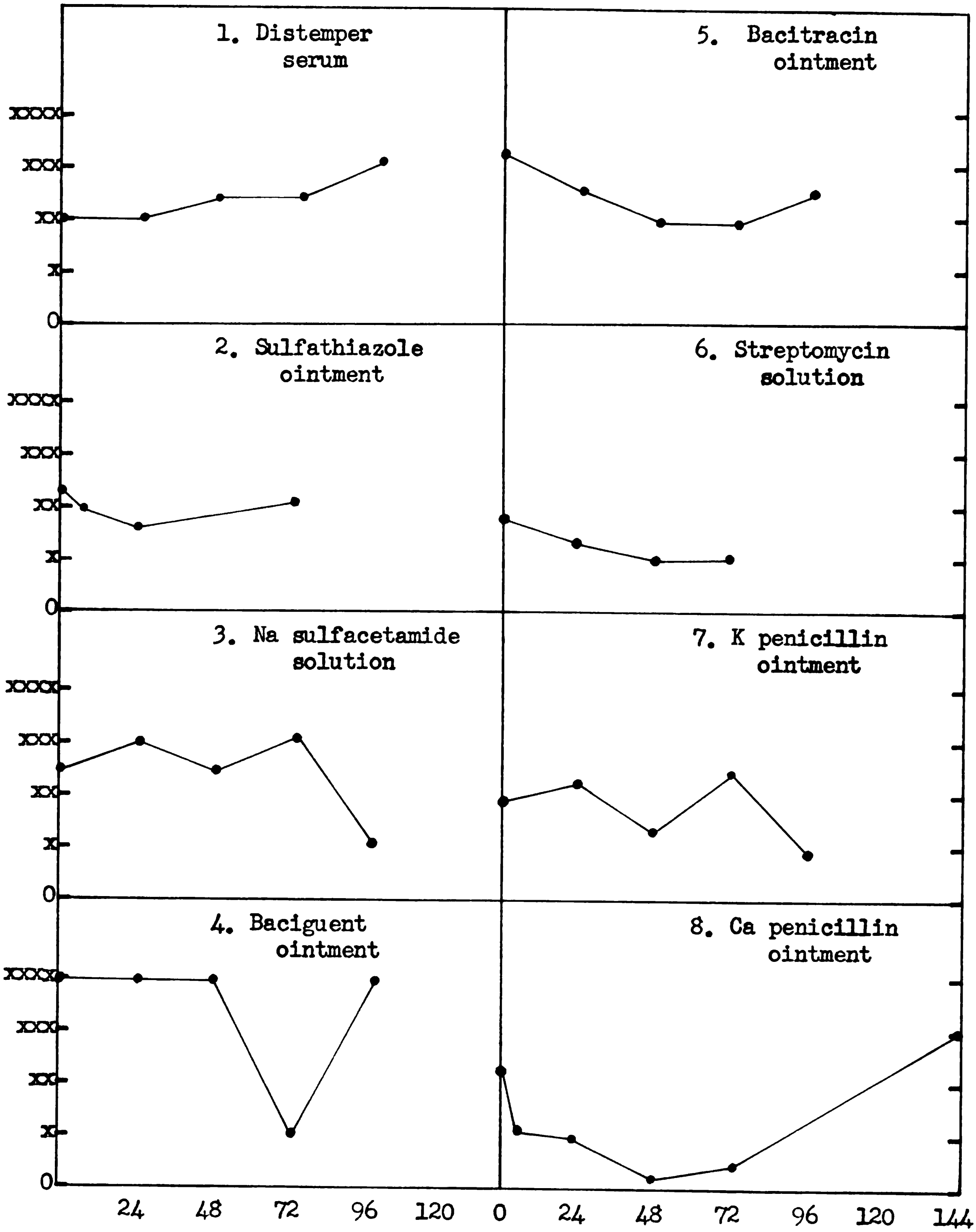
Figure 9 EFFECT OF TREATMENT WITH CALCIUM PENICILLIN OINTMENT ON BACTERIAL FLORAE IN CONJUNCTIVAL AND NASAL DISCHARGES



Hours after treatment

Note: xxxx numerous colonies
xxx less than 500 colonies
xx less than 100 colonies
x 1-20 colonies
0 no growth

Figure 10 EFFECTS OF TREATMENT WITH VARIOUS THERAPEUTIC AGENTS



Hours after treatment

(legend see figure 9)