

A STUDY OF THE BACTERIAL FLORA
OF THE UPPER RESPIRATORY TRACT OF
THE DOG AND CAT.

by

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C O N T E N T S

	Page
INTRODUCTION	1
<u>P A R T I - D O G</u>	
HISTORICAL SURVEY	5
SOURCE OF MATERIAL AND METHOD OF COLLECTION	26
TECHNIQUE AND PROCEDURE	32
RESULTS -	40
a. Staphylococci	47
b. Haemolytic streptococci	64
c. Non-haemolytic streptococci	82
d. Neisseria (Gram-negative cocci)	88
e. Haemophilus bronchisepticus	94
f. Coliform bacilli	99
g. Diphtheroid bacilli	102
h. Anthracoides (Bacillus Subtilis group)	106
i. Bacterium alkaligenes	109
j. Bacillus of Friedländer	114
k. Haemophilus canis	118
l. Proteus sp.	122
m. Leptotrichia sp.	126
n. Unidentified	134
DISCUSSION	148
SUMMARY	179

Contents

P A R T II-- C A T

	Page
HISTORICAL SURVEY	185
MATERIAL, TECHNIQUE AND PROCEDURE	189
RESULTS	190
DISCUSSION	206
SUMMARY	214
ACKNOWLEDGMENTS	216
REFERENCES	217

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INDEX OF TABLES

PART I - DOG

Table	Page
Ia. Description of the normal dogs	29
Ib. Description of the sick dogs.	31
IIa. Absolute and relative frequencies of various organisms in the nose, nasopharynx and tonsils of normal dogs	42
IIb. Absolute and relative frequencies of various organisms in the nose, nasopharynx and tonsils of sick dogs	44
III. Summary of the incidence of different organisms in normal and sick dogs	45
IV. Summary of the incidence of different organisms in the nose, nasopharynx and tonsils	46
V. Percentage carrier rates for Staphylococci in relation to Coagulase-positive strains	52
VI. The frequency of Staphylococci in the nose, nasopharynx and tonsils	52
VII. Summary of the characteristics of Staphylococci isolated from normal dogs	55
VIII. Summary of the characteristics of Staphylococci isolated from sick dogs	56
IX. Summary of the characteristics of Staphylococci isolated from normal and sick dogs (combined)	57

Index of Tables.

Table	Page
X. Comparison between the coagulase-positive and coagulase-negative strains of <i>Staphylococci</i>	59
XI. Characteristics of <i>Staphylococci</i>	61
XII. Percentage carrier rates for haemolytic streptococci.	67
XIII. The frequency of haemolytic streptococci in the nose, nasopharynx and tonsils.	67
XIV. Number of strains and groups of haemolytic streptococci from normal and sick dogs (combined).	69
XV. Number of strains and groups of haemolytic streptococci from normal dogs.	70
XVI. Number of strains and groups of haemolytic streptococci from sick dogs.	70
XVII. The incidence of different groups of haemolytic streptococci in normal and sick dogs.	71
XVIII. Biochemical reactions of group G strains of haemolytic streptococci	75
XIX. Biochemical reactions of group M strains of haemolytic streptococci.	77B
XX. Biochemical reactions of group C strains of haemolytic streptococci	78
XXI. Biochemical reactions of group L strains of haemolytic streptococci	79

Index of Tables

Table	Page
XXXIV. The frequency of Coliform bacilli in the nose, nasopharynx and tonsils	100
XXXV. Biochemical reactions of Coli- form bacilli	101
XXXVI. Percentage carrier rates for Diphtheroid bacilli in normal and sick dogs.	103
XXXVII. The frequency of Diphtheroid bacilli in the nose, nasopharynx and tonsils	105
XXXVIII. The frequency of B. subtilis group in normal and sick dogs.	107
XXXIX. Biochemical reactions of B. subtilis group	108
XL. Percentage carrier rates for Bacterium alkaligenes in normal and sick dogs.	110
XLI. The frequency of Bacterium alka- ligenes in the nose, nasopharynx and tonsils	110
XLII. Biochemical reactions of Bacterium alkaligenes	112
XLIII. The incidence of Friedländer's bacillus in the upper respiratory tract of dogs	116
XLIV. The frequency of Friedländer's bacillus in the nose, nasopharynx and tonsils	116

Index of Tables

Table	Page
XLV. Biochemical and other characteristics of Friedlander's bacillus	117
XLVI. Percentage carrier rates for H. canis in normal and sick dogs	121
XLVII. The frequency of H. canis in the nose, nasopharynx and tonsils	121
XLVIII. Biochemical reactions of Bacillus proteus	123
XLIX. Percentage carrier rates for Proteus bacilli in normal and sick dogs	125
L. The frequency of Proteus bacilli in the nose, nasopharynx and tonsils	125
LI. Percentage carrier rates for Leptotrichia sp. in normal and sick dogs	133
LII. The frequency of Leptotrichia sp. in the nose, nasopharynx and tonsils	133
LIII. Percentage carrier rates for unidentified group (a) organisms in normal and sick dogs	137
LIV. The frequency of unidentified group (a) in the nose, nasopharynx and tonsils	137
LV. Percentage carrier rates for unidentified group (b) in normal and sick dogs	141
LVI. The frequency of unidentified group (b) in the nose, nasopharynx and tonsils	141

Index of Tables

Table	Page
LVII. Percentage carrier rates for unidentified group (c) in normal and sick dogs	145
LVIII. The frequency of unidentified group (c) in the nose, nasopharynx and tonsils	145
LIX. Biochemical reactions of unidentified group (c)	146

(P A R T II - C A T)

LX. Absolute and relative frequencies of various organisms in the nose, nasopharynx and tonsils	191
LXI. Characteristics of Staphylococci	193
LXII. Biochemical reactions of haemolytic streptococci (group G)	194
LXIII. Characteristics of the "Influenza group bacilli"	197
LXIV. Biochemical reactions of Pasteurella septica	200
LXV. Biochemical reactions of non-haemolytic streptococci	202

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INTRODUCTION

The scarcity of information concerning the normal bacterial flora of the mouth and upper respiratory tract of the various domesticated animals has been the subject of recent comment by Veterinary Surgeons, arising out of queries regarding the possible carrier rate amongst animals of organisms pathogenic to those animals and also, in the case of pet animals, as to the possibility of their carrying and transmitting infections to their owners.

In 1941, Francis, while summarising the Public Health Report on the study of the bacterial flora of the nasopharynx of individuals of the population in London and South-East England (Straker, Bedford, Lovell and Rosher, 1939), drew the attention of veterinary research workers to the fact that there had been no adequate investigation of the bacteria normally present in the nasopharynx of any species of domestic animals, or of the factors which affected the carrier rate. He pointed out that a study of such a nature would be of obvious value in several of the respiratory diseases of domestic animals, particularly of those affecting young animals kept under intensive conditions. Bosworth (1947) quoted the need for an adequate survey of staphylococci in dogs in normal health and sickness, while Levi (1946) thought/

thought that a detailed study of the normal inhabitants of the nasopharynx of the cat would be of value in interpreting bacteriological findings in cases of "snuffles". Much has been said in recent years about the incidence of haemolytic streptococci in dogs and their pathogenicity (Pilot, Bück, Davis and Eastman, 1936; Hare and Fry, 1938; Stafseth, 1940; Hare, 1946; and Garside, 1947), but the frequencies of these organisms in relation to other possible pathogens have not been studied.

Taking these factors into consideration, it was decided to make a general survey of the various organisms present in the upper respiratory tracts of dogs and cats. These animals were chosen because of the facilities available for the collection of material (Clinical Department attached to the Royal (Dick) Veterinary College, Edinburgh), but an important factor which weighed too in selecting these two species of animals, was the Public Health aspect. It was thought that the results of this investigation would provide information as to whether or not these animals were frequent carriers of human pathogens. In modern society the dog and cat have become such household pets that they are fairly considered as members of the family, having free access practically to everything and to every place in their masters' homes, so that if these animals harbour any of the human pathogens, transmission to human beings may be easily accomplished. Several reports regarding the high frequency of haemolytic/

haemolytic streptococci in the throats of dogs, as stated above, repeated reports in the medical literature of Pasteurella infection in man following cat bites and, in one instance, dog bite (Kapel and Holm, 1930; Schenk, 1938; Allin, 1942; Allott et al, 1944; and Hansman and Tully, 1945), and the cases recorded of tularaemia following cat bites (Smiles, 1931; Collins, 1933-34) specially influenced this aspect of the study.

HISTORICAL SURVEY

Early information as to the bacterial flora of the nasopharynx of the dog all accrued as a result of investigations into the aetiology of canine distemper.

Millais (1890), during his experiments to discover the pathogenic micro-organisms of distemper, studied eight young dogs affected with the disease and cultivated from the nasal discharges a long bacillus which liquefied gelatin, the growth depositing itself as a whitish scum. Finally, when the whole of the gelatin was liquefied, flakes gathered at the base and, if stirred, appeared to be of a ropy consistency. Along with the bacillus he also found a micrococcus distinguished by its ability to liquefy gelatin in all cases that died of distemper with pneumonic complications. This organism, he found in the lungs, liver, spleen, trachea and nasal mucosa, and he presumed it to be the cause of the lung lesions, while the two organisms combined caused pneumo-distemper. He claimed to have produced distemper in two young dogs by syringing the nasal fossae with cultures of his bacillus, but he did not describe his organisms in any more detail.

The next worker in this field was Galli-Valerio (1896, 1908), who cultivated a motile, oval bacillus from the respiratory tract, brain and spinal/

spinal cord of dogs which had died of distemper. On cultivation, this organism produced gas in the gelatin stab with liquefaction and did not coagulate milk. It formed a dense turbidity in peptone broth at 18° to 29°C. in 24 hours and, after some days' incubation, white flocculi appeared at the bottom of the tube. It grew readily on potato, but had no action on any of the carbohydrates and did not form indole.

In his first paper, Valerio stated that his organism was Gram-positive and that it had a small spore, but subsequently he retracted his statement and recorded that it was Gram-negative and had no spore. The spores erroneously observed, he said, were involution forms.

Inoculations with cultures of this organism subcutaneously, intravenously and directly into the lungs of dogs from five to eight years old neither produced symptoms of distemper nor any other severe reaction, whereas cultures injected into a young dog about six months old produced the disease, with typical symptoms, in eleven days and caused the death of the animal nineteen days after the inoculation.

Two young dogs placed in the same cage in which these dogs had been kept, and which had not been disinfected, became infected and showed typical symptoms of distemper.

In his paper on the bacteriology of distemper, Jess (1899) stated that he had cultivated from the nasal secretion of infected dogs a Gram-positive, motile, small bacillus measuring $1.8 - 2.3 \mu \times 0.6 - 0.9 \mu$

producing dense turbidity in broth with pellicle formation, and forming a dull grey film on agar. He found this organism pathogenic to dogs, cats and guinea pigs, and he was able to produce distemper experimentally with the culture of this organism.

Copeman (1900) isolated from the nasal secretion, tracheal mucosa and exudation from the lungs of dogs died of distemper, a small Gram-negative cocco-bacillus which in smears from broth cultures not infrequently formed chains, sometimes of considerable length. It grew readily on surface agar at 36°C . forming greyish, glistening, circular colonies. In broth it caused at first general turbidity and later granular deposit at the bottom of the tube, leaving the supernatant fluid clear. It did not coagulate milk. On potato it grew with difficulty, but after some days' incubation a moist-looking streak of a pale buff colour was observed. Growth in gelatin was slow without liquefaction.

One cubic centimeter of the broth culture injected subcutaneously into a dog weighing 7 kilos induced an attack of distemper which terminated fatally in about a week after the inoculation. He claimed/

claimed to have conducted such experiments on several dogs.

In 1901, 1906, and again in 1907, Lignières claimed to have isolated from the nasal discharge as well as the blood and internal organs of dogs, generally in the early stages of distemper, an organism which belonged to the Pasteurella group. He described it as a small Gram-negative, non-motile, coccobacillus which readily assumed pleomorphic and involution forms. It did not grow on potato, formed no indole, produced no change in milk, and had no action on any of the carbohydrates. It did not liquefy gelatin. In neutral or slightly alkaline broth, it grew better, for acidity prevented its growth. Broth cultures did not show the characteristic properties of the Pasteurella - a uniform turbidity - but produced instead flocculi formation with clear supernatant fluid; a small amount of serum added to the broth improved the growth.

Subcutaneous injection of mice with 0.25-0.5 ml. of broth culture often had no effect, causing at most a swelling at the site of injection. In other cases death occurred after two to four days. Intra-peritoneal injections were lethal within twenty-four hours. One cubic centimeter of broth culture inoculated intraperitoneally killed guinea pigs within twenty-four hours. Five cubic centimetres subcutaneously/

subcutaneously killed them in two days. Rabbits died within twenty-four to forty-eight hours after intravenous, subcutaneous or intraperitoneal injection of 1 c.c. broth culture. From his experimental inoculation of this bacillus into susceptible dogs, Lignières concluded that the more typical symptoms of distemper, such as gastroenteritis, erythema, pericarditis and pneumonia, resulted when the organism was introduced intravenously. Subcutaneous inoculation into young dogs of one to two cubic centimeters of broth culture induced within twenty-four hours a severe local edematous swelling with, later, the formation of pus and frequently a fatal issue through the development of septicaemia. By placing sick and healthy dogs in contact, the latter were infected. The disease could not be produced by feeding animals on milk to which pure cultures of the organism had been added.

Lignières also reported the isolation on two occasions from the lung lesions of distemper dogs of *Bacillus ozaenae foetidus* of Perez, which he thought was the cause of pneumonic complications, while the *Pasteurella* organism was the primary invader. He did not, however, confirm this theory to any degree of satisfaction.

Von Wunschleim (1905) also in his investigation into the aetiology of distemper obtained from the nasal/

nasal secretion, heart blood and exudate from spleen and kidneys of dogs which had died of distemper, a Gram-negative, non-sporing, non-motile short bacillus $0.75 - 1.5 \mu \times 0.3 - 0.5 \mu$ with rounded ends which exhibited polar staining.

The growth on agar was white, diffuse and opalescent. In broth there was diffuse turbidity and often pellicle formation. On potato brownish growth was seen. Milk was not clotted, indole was not formed and gelatin was not liquefied. The action on carbohydrates was not stated.

The organism was pathogenic to mice, rats, guinea pigs, hens, rabbits, pigeons, dogs, and cats. The author claimed to have produced the clinical picture of the catarrhal and nervous forms of distemper by inoculation of the culture into susceptible dogs. He believed that the organism belonged morphologically, biologically and culturally to the group causing haemorrhagic septicaemia and so he called it *Bacillus canicidus*.

Heuer (1906) isolated from the nasal discharges, lungs and heart blood of dogs dead of distemper, *Staphylococcus aureus* and *albus* and a short, small, non-motile Gram-negative bacillus producing a fine white growth on agar, slightly clouding broth with granular sediment and stringy masses. The organism did not liquefy gelatin; its growth on potato was very poor and it produced only slightly acid reaction in litmus milk.

Heuer, however, studied only three dogs and, further, the animal experiments he conducted were too few to warrant any conclusion as to the identity and pathogenicity of the organism.

The descriptions of the recovered organisms given by these early workers are too inadequate to allow of any definite conclusions regarding the species to which they belong.

The organism of Jess compares favourably with *B. bronchisepticus* in its morphology and other characteristics, except for its Gram-positive staining, but it is possible that he too had committed the same mistake as Valerio (loc. cit.), who also stated at first that his organism was Gram-positive, but subsequently recorded it as Gram-negative.

It is difficult to assess the claims of Lignieres and Von Wunschleim that their organisms were of the *Pasteurella* type, as their cultural and even the biochemical characteristics given in one case are not satisfactory to group them as such. Further detailed study on similar lines made subsequently by other workers never gave any indication of the presence of haemorrhagic septicaemia organisms in distemper cases.

In his extensive study of canine distemper, with special reference to its bacteriology, Ferry (1910, 1911, 1912) recorded the isolation of *Bacillus bronchisepticus* from the respiratory tracts of eighty-six dogs out of a total of ninety-three affected with distemper/

distemper, and studied the organism in detail. At first he named this organism *Bacillus bronchicanis*, but subsequently he found that the same organism was the cause of a severe infection among other laboratory animals. In an epizootic which destroyed many guinea pigs, a number of rabbits and monkeys, this same bacillus was isolated, generally in pure culture, from the respiratory tract and frequently from the blood. In view of the isolation of this bacillus from the blood in 26.5 per cent. of his cases of canine distemper and in 33.7 per cent. of the fatal cases among other laboratory animals, he changed the name of this organism to *Bacillus bronchisepticus*.

Ferry described the organism as a Gram-negative, non-sporing, short, narrow bacillus, usually found singly, but often in pairs. In liquid media it sometimes appeared in long chains. It did not stain well by Gram's stain, but stained best with Löffler's methylene blue, with which characteristic bipolar staining occurred. The organism was "active and progressively motile". On agar plates after twenty-four hours at 37°C. the colonies were exceedingly small, not much larger than a pinpoint, translucent and slightly raised. After forty-eight hours they had increased to pinhead size, were round, convex, smooth, amorphous and translucent. After seven days the colonies were much larger and thicker with a grumose/

grumose centre and undulate edge. The bacillus grew readily at room temperature in gelatin stab, but caused no liquefaction of the medium. It had no action on any of the carbohydrates and it produced no indole. Its distinctive cultural characteristics were especially revealed when grown on litmus milk and on potato. It caused no change in the milk medium after twenty-four hours' incubation at 37°C. but after seventy-two hours the upper half of the medium assumed a deeper blue colour which extended through the whole tube in five days. After fourteen days the colour had entirely disappeared from the bottom of the tube, and in the upper portion had become a darker blue. On potato medium after twenty-four hours' growth at 37°C. a rather thick raised growth appeared, which was sticky and light tan in colour and emitted a decided stale odour. After forty-eight hours the growth had become thicker, shiny and a darker tan, with a darkening of the medium. Sera from dogs with distemper always agglutinated this bacillus, while that from normal dogs contained no specific agglutinin.

Ferry was satisfied that he had produced the disease experimentally by the inoculation of living cultures of this bacillus into susceptible dogs. Also, by injecting cultures into the trachea of puppies he was able to produce in several instances the classical symptoms and lesions of distemper.

Ferry/

Ferry claimed that this bacillus was the primary and essential aetiological factor in canine distemper.

One-fourth to one whole agar culture of the organism, when injected subcutaneously into cats, dogs, rabbits, guinea pigs, rats, mice and pigeons, produced death only of mice, but when injected intraperitoneally into these animals, all died, except the rabbits, in one to three days with purulent peritonitis, the organism being recovered in all cases from the peritoneal exudate and respiratory tract, and in a few cases from the heart blood, liver and spleen.

Ferry reported that he had killed and autopsied a large number of normal dogs, but he was able to recover the organism only from three dogs, which he presumed had been exposed to natural distemper. He was of the opinion that *B. bronchisepticus* was not the normal inhabitant of the respiratory tracts of dogs.

Entirely independently of Ferry, this same bacillus was isolated from cases of distemper in dogs by McGowan (1911, 1912). In an epizootic characterised chiefly by catarrhal respiratory symptoms and occurring among a considerable number and variety of animals in the laboratories of the Royal College of Physicians, Edinburgh, this investigator isolated a bacillus culturally and biologically identical with *B. bronchisepticus* of Ferry. The disease/

disease, as it occurred in dogs, was characterised by distemper-like symptoms, such as purulent discharge from the eyes and nose, coughing and vomiting, diarrhoea, fever, abdominal rash, emaciation, paralysis and chorea. Cats were especially susceptible to the disease and were found dead or dying with catarrhal symptoms. From thirty-two out of forty-two dogs and from sixty-five out of sixty-seven cats showing symptoms, he isolated this bacillus without difficulty from the mucopurulent nasal discharge, the trachea or lungs, but not from the blood. He also recovered it from sixteen rabbits, seven guinea pigs, a goat, a monkey and two ferrets, most of which showed some symptoms of respiratory catarrh. A laboratory assistant who was constantly handling rabbits and guinea pigs and had suffered for over a year with a severe nasal catarrh proved to be harbouring this bacillus in pure culture in the nose, but attempts to recover this bacillus from ten other cases of catarrh among laboratory workers proved fruitless. McGowan also recorded two cases of experimental distemper induced by the introduction of cultures of his bacillus into the nasal passages of susceptible dogs, in one of which a severe attack of distemper occurred, ending with convulsions and paralysis. He subsequently isolated the same organism from the nose of one of the five laboratory dogs, presumably/

presumably in contact with infection, but failed to find it in any one of nine dogs from outside sources.

Torrey and Rahe (1913) studied about one hundred and fifty dogs of which ninety were known to have been infected with distemper and which were in various stages of the disease or dead of the infection. They were able to isolate *B. bronchisepticus* from the nasal discharge, lungs, larynx and trachea from sixty-five of these animals. Of the sixty-five positive cases, the organism was also present in the liver in 30 per cent., in the spleen in 20 per cent., in the kidney in 16 per cent., and in the blood in 6 per cent. of cases. These workers were also able to induce distemper in susceptible dogs by experimental inoculations and by blowing the dried organisms in infected dust into the nasal passages.

The strains of these organisms isolated from sixty-five cases revealed an absolute uniformity in cultural reactions, except in their reaction to one test; only 13 per cent. of the strains reduced nitrates to nitrites, while 87 per cent. had no reducing action.

While Ferry claimed the isolation of this organism from the blood of about 30 per cent. of his positive cases, and while McGowan denied having found it in the blood in any instance, Torrey and Rahe recorded its isolation from the blood of about 5 per cent./

cent. of their positive cases. These workers agreed with Ferry that the trachea was the most probable seat of infection, but they did not underestimate the importance of the presence of this bacillus in the nasal discharge, even though they were unable to agree with McGowan that the infection was primarily in the nose.

Torrey and Rahe also made an effort to investigate the presence of *B. bronchisepticus* in the tissues of normal dogs. For this purpose they selected apparently healthy and normal dogs and divided them into three groups:-

- a. Those which were known not to have been exposed to distemper.
- b. Those whose history was not known.
- c. Those whose immunity to the infection had been proved by exposure.

Two puppies classified under the first group, which had never shown any symptoms of distemper and which had been carefully isolated for ten weeks before being killed, did not show the presence of *B. bronchisepticus* in the cultures made from different parts, such as nose, trachea, lungs, blood, liver, spleen, etc. Three dogs belonging to the second group were similarly studied, but from none of them was the organism isolated. Twelve dogs belonging to the third group were studied; only three of them harboured this bacillus, in two cases in the respiratory/

tory tract and in one instance in the spleen alone.

So, Ferry, McGowan, Torrey and Rahe, working independently and contemporaneously, arrived at the conclusion that *B. bronchisepticus* was the primary aetiological factor in distemper in dogs, cats, rabbits, ferrets, guinea pigs, and monkeys, and it was found predominantly in the respiratory tracts of such animals. (Ferry, 1915). Further, both H. per-

This view was not shared by Hardenburgh (1925), who studied the importance of *B. bronchisepticus* in relation to distemper, and after a dispassionate survey of the claims made for it, coupled with the results of his own extensive and careful experiments, was unable to regard it as the primary aetiological agent, a conclusion vindicated by the brilliant work of Dunkin and Laidlaw (1926) and Laidlaw and Dunkin (1928), who proved beyond doubt that the primary cause of distemper was a filtrable virus, whilst *B. bronchisepticus* was a secondary infective agent responsible for the involvement of the lungs.

This organism has in recent years been included in the *Brucella* group, whose type species, *Br. Melitensis*, it resembles in both individual and colonial morphology and in its inability to ferment carbohydrates. Wilson and Miles (1946), however, prefer to include it in the genus *Haemophilus*, at least provisionally, in view of its conspicuous degree of antigenic/

antigenic similarity to *Haemophilus pertussis* and to its toxin production rendering it capable of producing lesions in guinea pigs similar to those produced by *H. pertussis* in rabbits and puppies (Mallory, Horner, and Henderson, 1912; Smith, 1913), while the lesions produced by *B. bronchisepticus* in the lungs of rabbits are similar to the lung lesions of human whooping cough (Rhea, 1915). Further, both *H. pertussis* and *B. bronchisepticus* are natural parasites of the upper respiratory tract.

Referring to other organisms in the upper respiratory tract apart from *H. bronchisepticus*, Hamilton Kirk (1922) records that according to the information received by him from the superintendent of a well-known English research laboratory (name not given), the organisms isolated from the nasal discharge of a large number of cases of distemper in dogs were one or more of the following: *Staphylococcus aureus* and *albus*, *Bacterium coli communis*, *Pneumococcus*, *Streptococcus maximus*, and *Bacillus bronchisepticus*.

According to Brumley (1938), the most common organisms found in the tonsils of dogs and cats are streptococci and staphylococci, which multiply rapidly in the tonsils, bringing about tonsillitis. Severe forms of pharyngitis are occasionally seen in week-old puppies and kittens, in some cases amounting almost/

almost to an enzootic affecting the entire litter. The most common organisms found in such cases are streptococcus and Bacillus necrophorus.

Perez (1901, 1913), while engaged in the study of ozaena in human patients at the Pasteur Institute of Paris, was informed by Lignières (loc. cit.) that on two occasions he had isolated from the respiratory tract of dogs affected with distemper the cocco-bacillus foetidus ozaenae, which Perez had isolated from Ozaena patients and thought to be the cause of that malady. Being interested in this information, Perez studied the nasal cavities of other animals, such as horses, donkeys, pigs, sheep, monkeys, poultry, fish, and frogs, but did not find the organism in any of them. He isolated it only in the saliva and nasal mucosa of one normal dog out of the six he studied. He claimed that dogs were the carriers of this organism in the saliva and nose, and that they, particularly sick ones in which these organisms were found in predominant numbers, were highly infective to man. He did not state the number of healthy and sick dogs he studied in all.

The Cocco-bacillus foetidus ozaenae of Perez (Perez's bacillus) is a small Gram-negative, sluggishly motile organism, growing in broth with characteristic nauseating odour. It produces indole in peptone water and ferments only glucose with the production of acid and gas. It has now been/

been assigned to the proteus group, even though it does not liquefy gelatin. It is found associated with ozaena, but it is doubtful whether it is the primary causal organism of that disease (Mackie and McCartney, 1948 a.).

In 1936, Pilot, Buck, Davis and Eastman, in their investigations into the relationship of the haemolytic streptococci to tonsillar infection and the incidence of similar streptococci in the tonsils of normal dogs, recorded that haemolytic streptococci were constantly present in the tonsils of apparently healthy dogs. They occurred in predominant numbers in acute tonsillitis and were also secondary invaders in the broncho-pneumonia of distemper. Experimentally, the organisms were capable of producing acute tonsillitis when freshly isolated strains were swabbed on to the surface of dogs' tonsils. A human strain (*Streptococcus epidemicus*), on the other hand, failed to produce a reaction. The authors were of the opinion that the streptococci from dogs conformed to the animal type and differed from the human ones. The same authors, by examining the excised tonsils of fourteen adult dogs and tonsillar swabs from thirteen young dogs, found 77.7 per cent. of the tonsils carrying haemolytic streptococci, while the positive cases were very high (92.3 per cent.) in young dogs when considered separately. The strains were neither definitely identified nor grouped.

Eastman (1936) also recorded the occurrence of tonsillitis in dogs with manifestations of enlarged tonsils, often with exudate and fever, and found the cause to be haemolytic streptococci. These strains also were not grouped.

Hare and Fry (1938, a & b) in their comprehensive survey of the infection of dogs by beta-haemolytic streptococci, while describing various syndromes caused by these organisms, such as adenitis, sterility, septicaemia, etc., stated that beta haemolytic streptococci were also responsible for tonsillitis and pharyngitis in adult dogs and puppies. According to these authors, the strains isolated from dogs belonged mainly to Lancefield's serological groups G and C, while strains from groups A, B, D, and E were occasionally met with. On one occasion the authors had isolated from 128 bitches, dogs and whelps beta-haemolytic streptococci of the following groups:

88 group G; 20 group C; 5 group A; 4 group D; 2 group E, and 9 undetermined.

Valsberg and Patchogue (1938) also recorded a case of acute haemolytic streptococcal tonsillitis in a two-and-a-half year old Scottish terrier bitch with secondary staphylococcus infection involving the skin, middle ears, foot pads and vagina.

In 1940, Stafseth isolated haemolytic streptococci from the tonsils of five young cocker spaniel puppies/

puppies showing severe tonsillitis with mucopurulent discharge from the crypts, and also examined the swabs from the tonsils of five apparently healthy puppies in the same kennel, finding that three of them harboured haemolytic micrococci, one, haemolytic streptococci, and one, both of these organisms. These strains also were not grouped.

Minett and Ellis (1940) studied streptococcal infections in dogs with special reference to genital infections in bitches and the ensuing mortality in puppies, and recorded the presence of these organisms not only in the vagina and milk of bitches but also in the tonsils and prepuce of the male. The majority of the strains isolated fell into group G and a few fell into group C.

Hare (1946) studied four hundred cases of infection with beta-haemolytic streptococci in dogs extending over a period of ten years. He frequently isolated these organisms from the tonsils and pharynx of whelps which had died within three days after birth, and also from the tonsils of adult dogs with tonsillitis. Most of the strains isolated belonged to Lancefield's group G and a few to group C.

Hare does not agree that beta-haemolytic streptococci are recoverable from the tonsils of healthy dogs, but is of the opinion that such positive cases are the cyclical waning phases of chronic infection.

In his study of the haemolytic streptococci in various species of domesticated animals, Garside (1947) recorded the results of his two surveys of the incidence of haemolytic streptococci in the throats of the general dog population. In one, the examination of the tonsils of one hundred dogs attending the out-patient clinic of the Royal (Dick) Veterinary College revealed 34 per cent. positive cases. In another survey at a different time of the year, a similar number coming from the clinic of a private practitioner in Edinburgh were examined, and 60 per cent. were found positive. A few of these strains were grouped and found to belong to group G.

Bosworth (1947), discussing the question as to whether the presence of staphylococci in the tonsils of dogs could be held responsible for chronic ill-health associated with mild widespread pustular dermatitis, stated that the records of the Royal Veterinary College, London, indicated that staphylococci were not present as commonly in tonsil swabs as streptococci, but they were occasionally met with. Haemolytic strains of staphylococci had been isolated from throat swabs of dogs with enlarged pharyngeal and cervical lymphatic glands, from the throat swabs of a dog with furunculosis, and also occasionally from throat swabs in cases in which it seemed/

seemed unlikely that the tonsil was playing any part in the disease observed. Bosworth admitted that no adequate survey of staphylococci in dogs in normal health and sickness had yet been made, and in the absence of such a study he was of the opinion that it was premature to attach significance to the mere presence of staphylococci on the tonsils, but it was unwise to disregard them when they occurred in an obviously septic focus accompanied by the involvement of the neighbouring lymphatic glands.

The remaining twenty-five were classified as sick dogs with one of the following complaints: nasal catarrh, bronchitis, convulsions, chorea, spasms and debility, mostly associated with distemper. Care was taken not to select for study any dog which had received treatment in any form.

All the dogs were made available at the out-patient clinic of the Royal (Dick) Veterinary College. Sixty-seven (50 normal and 17 sick) were destroyed painlessly at the request of the owners, either by the intravenous injection of saturated solutions of potassium chloride or by the intracardial injection of potassium. These destroyed dogs were preferred for examination for the obvious advantages that the parts could be well rubbed and a good quantity of mucus made available for examination. Further, it was found not practicable by any route to wash the nasopharynx of a living dog satisfactorily. It was a great improvement.

SOURCE OF MATERIAL AND METHOD OF COLLECTION

This part of the study comprised the examination of the upper respiratory tracts of one hundred dogs of different breeds and of different ages varying from two months up to fourteen years, as shown in Table Ia and Ib. Seventy-five of these animals were for all practical purposes considered normal or had at least no visible signs of any respiratory troubles, while the remaining twenty-five were classified as sick ones with one of the following complaints: nasal catarrh, bronchitis, convulsions, chorea, anaemia and debility, mostly associated with distemper. Care was taken not to select for study any dog which had received treatment in any form.

All the dogs were made available at the out-patient clinic of the Royal (Dick) Veterinary College. Sixty-seven (50 normal and 17 sick) were destroyed painlessly at the request of the owners, either by the intravenous injection of saturated solutions of magnesium sulphate or by the intracardial injection of nembutol. Such destroyed dogs were preferred for examination for the obvious advantages that the parts could be well rubbed and a good quantity of mucus made available for examination. Further, it was found not practicable by any device to swab the nasopharynx of a living dog satisfactorily. Even a swab improvised/

improvised on the pattern of West's post-nasal swab used for swabbing the human nasopharynx was not of much avail in dogs, as it was difficult to keep the mouth of the dog sufficiently well opened to conduct this operation. It was possible to take nostril swabs of quiet dogs, but to swab the nose of a vicious or nervous dog was difficult without using some force, attended by the risk of injuring the mucous membrane of the nasal cavity, resulting in haemorrhage, which would have been resented by the owners. Swabbing of the tonsils was fairly easy, but here too some amount of force had to be used in nervous dogs. All these difficulties were overcome by choosing destroyed dogs, and by that means more accurate results were expected. In the case of thirty-three (25 normal and 8 sick) only nose and tonsils were studied.

As soon as the dog was destroyed, both nostrils and the surrounding skin were thoroughly wiped with cotton wool soaked in absolute alcohol. Then a sterile cotton wool swab was passed through one nostril into the nasal cavity as far as possible, withdrawn and passed into the other nasal cavity in the same way. Then the mouth of the dog was kept wide open by an assistant, the tongue was pressed down, exposing both the tonsils, which were rubbed with a second swab. Again a third swab, which was slightly bent at the tip, was inserted behind the soft palate and/

TABLE Ia.

Description and general condition of the seventy-five apparently normal dogs examined.

Dog No.	Breed	Sex	Age	Living or Destroyed	General condition	Visible signs of respiratory trouble
1	Cocker spaniel	Female	6 months	Destroyed	Fracture, femur	None
2	Cocker spaniel	Female	14 years	Destroyed	Normal	None
3	Collie, cross	Female	10 years	Destroyed	Very fat	None
4	Cocker spaniel	Male	5 years	Destroyed	Slight skin disease	None
5	Mongrel	Male	1 year	Destroyed	Normal	None
6	Collie, cross	Female	13 years	Destroyed	Normal	None
7	Scottish terrier	Male	4 years	Destroyed	Normal	None
8	Mongrel	Male	2 years	Destroyed	Normal	None
9	Cocker spaniel	Male	5 years	Destroyed	Normal	None
10	Greyhound	Female	8 years	Destroyed	Fractured Tibia	None
11	Terrier, cross	Male	6 months	Destroyed	Normal	None
12	Terrier, cross	Female	3 years	Destroyed	Normal	None
13	Terrier, cross	Female	12 years	Destroyed	Very fat	None
14	Collie	Male	14 years	Destroyed	Debilitated	None
15	Collie	Male	14 years	Destroyed	Injured eye	None
16	Terrier; wire-haired	Male	11 years	Destroyed	Normal	None
17	Terrier, cross	Male	1 year	Destroyed	Normal	None
18	Cocker spaniel	Male	1 year	Destroyed	Normal	None
19	Terrier, cross	Male	7 years	Destroyed	Normal	None
20	Terrier, cross	Male	14 years	Destroyed	Cataract, both eyes	None
21	Alsatian	Male	3 years	Destroyed	Fracture, hip	None
22	Terrier, cross	Female	6 years	Destroyed	Eczema	None
23	Terrier, cross	Female	5 months	Destroyed	Normal	None
24	Spaniel	Female	5 years	Destroyed	Eczema	None
25	Spaniel	Male	8 months	Living	Fracture, hip	None
26	Labrador	Male	11 years	Destroyed	Normal	None
27	Terrier, wire-haired	Male	12 years	Living	Normal	None
28	Terrier, cross	Female	5 months	Destroyed	Normal	None
29	Terrier, cross	Male	1 year	Destroyed	Normal	None
30	Terrier, cross	Male	4 years	Living	Normal	None
31	Hound	Female	5 years	Destroyed	Normal	None
32	Collie	Female	6 months	Destroyed	Normal	None
33	Terrier, cross	Male	2 years	Living	Normal	None
34	Terrier, cross	Male	2 years	Destroyed	Weak and anaemic	None
35	Hound	Male	4 years	Destroyed	Compound fracture, tibia	None
36	Cocker spaniel	Male	2 years	Living	Normal	None
37	Hound	Male	6 months	Destroyed	Fracture, loin	None
38	Golden retriever	Female	8 years	Living	Normal	None

TABLE Ia (Contd.)

Dog No.	Breed	Sex	Age	Living or Destroyed	General condition	Visible signs of respiratory trouble
39	Spaniel, cocker	Female	3 years	Living	Normal	None
40	Spaniel	Female	5 years	Destroyed	Fracture, hip	None
41	Scottish terrier	Female	13 years	Destroyed	Normal	None
42	Terrier, cross	Male	2 years	Destroyed	Skin disease	None
43	Cocker spaniel	Female	13 years	Destroyed	Weak	None
44	Terrier, cross	Male	2 years	Living	Normal	None
45	Collie	Male	5 years	Living	Normal	None
46	Terrier, cross	Male	1 year	Destroyed	Normal	None
47	Terrier, cross	Male	18 months	Destroyed	Normal	None
48	Collie	Male	15 years	Destroyed	Weak	None
49	Alsatian	Male	5 months	Destroyed	Compound fracture, femur	None
50	Terrier, cross	Female	8 months	Destroyed	Normal	None
51	Scottish terrier	Male	3 months	Living	Normal	None
52	Terrier, cross	Male	13 years	Destroyed	Slight skin disease, weak	None
53	Alsatian	Male	6 months	Living	Slightly indisposed	None
54	Labrador	Male	2 years	Living	Normal	None
55	Alsatian	Male	2 years	Destroyed	Skin disease	None
56	Terrier, cross	Female	4 months	Living	Normal	None
57	Golden cocker	Female	2 years	Living	Normal	None
58	Golden cocker	Female	2 years	Living	Normal	None
59	Cocker spaniel	Male	2 months	Living	Normal	None
60	Cocker spaniel	Male	2 months	Living	Normal	None
61	Poodle	Female	2 years	Destroyed	Fracture, femur	None
62	Scottish terrier	Male	2 years	Destroyed	Fracture, hip	None
63	Terrier, cross	Male	3 years	Destroyed	Skin disease	None
64	Scottish terrier	Male	2½ years	Destroyed	Skin disease	None
65	Terrier, cross	Male	3 years	Destroyed	Fracture, tibia	None
66	Terrier, cross	Male	1 year	Destroyed	Eczema	None
67	Hound	Male	2 years	Living	Normal	None
68	Golden retriever	Male	11 years	Living	Normal	None
69	Collie	Female	14 years	Destroyed	Tumour on mammary gland	None
70	Terrier, cross	Male	2 years	Living	Wound	None
71	Cocker spaniel	Male	1 year	Living	Normal	None
72	Scottish terrier	Male	1 year	Living	Normal	None
73	Terrier, cross	Female	4 years	Living	Wound, neck & back	None
74	Cocker spaniel	Male	8 years	Living	Wound, foot	None
75	Terrier, cross	Male	5 years	Living	Skin disease	None

TABLE Ib

Description and general condition of twenty-five sick dogs

Dog No.	Breed	Sex	Age	Living or Destroyed	Symptoms of illness	Visible signs of respiratory trouble
1	Alsatian	Male	1 year	Destroyed	Watery discharge from nose and eyes. Chorea	Slight cough
2	Alsatian	Male	2 years	Living	Discharge from the eyes. Chorea.	None
3	Wire-haired terrier	Male	1 year	Destroyed	Chorea following distemper	None
4	Fox terrier	Male	1 year	Destroyed	do.	None
5	Cocker spaniel	Male	5 months	Living	Initial stage of distemper	Discharge from the nose
6	Terrier, cross	Male	6 months	Destroyed	Distemper	Discharge from the nose. Slight cough.
7	Hound	Male	1 year	Destroyed	Chorea	None
8	Fox terrier	Male	7 months	Living	Early symptoms of distemper.	Discharge from the nose.
9	Cocker spaniel	Male	1 year	Destroyed	Clear symptoms of distemper	Masal catarrh and cough.
10	Collie	Male	4 years	Living	Weak and debilitated	Inflamed tonsils and slight cough
11	Cocker spaniel	Male	1 year	Living	Ulceration of the mouth	None
12	Collie	Female	1 year	Living	Early symptoms of distemper	Cough
13	Spaniel, cross	Male	1½ years	Living	do.	Discharge from the nose and cough
14	Collie	Male	1 year	Living	Debilitated	None
15	Alsatian	Female	1 year	Destroyed	Epilepsy as a sequelae of distemper	None
16	Pomeranian	Female	1 year	Destroyed	Anaemic and debilitated	None
17	Alsatian	Male	1½ years	Destroyed	Chorea	None
18	Cocker spaniel	Female	8 years	Destroyed	Debilitated	None
19	Terrier, cross	Male	1 year	Destroyed	Early symptoms of distemper	Discharge from the nose and cough
20	Terrier, cross	Female	2 years	Destroyed	Convulsions	None
21	Cocker spaniel	Female	1 year	Destroyed	Convulsions	Slight discharge from the nose.
22	Terrier, cross	Male	2 years	Destroyed	Chorea	None
23	Scot. terrier	Female	2 years	Destroyed	Chorea	None
24	Greyhound	Male	2 years	Destroyed	Debilitated	None
25	Cocker spaniel	Female	1 year	Destroyed	Early symptoms of distemper	Slight watery discharge from the nose

TECHNIQUE AND PROCEDURE

Immediately after obtaining the swabs with the specimens of mucus, cultures were made on the following media and incubated at 37°C. aerobically:

1. Five per cent. horse blood agar plate.
2. Chocolate agar plate.
3. MacConkey agar plate.
4. One per cent. glucose broth.

The swab was rubbed over a small area at the edge of each plate and then successive stroke inoculations were made in the remainder of the plates by means of a platinum loop, the tip being charged several times from the area inoculated directly with the swab. After rubbing on the plate, the swab was dipped into a tube of glucose broth. Cultures from the nose, nasopharynx and tonsils were marked as N, NP, and T respectively, bearing also the date.

The plates were examined after 18 hours' incubation and the presence of different types of colonies and their approximate numbers were noted. The blood agar plate was examined particularly for the presence of colonies of haemolytic streptococci, and such colonies, when present, were subcultivated to another blood agar plate. Other prominent colonies were also subcultured on to blood agar plates. The subcultivation of streptococci at this stage was necessary, as it was found by experience that on longer/

longer incubation the whole medium became haemolysed owing to the activity of other organisms, such as staphylococci or coliform organisms, when it became difficult to pick up haemolytic streptococcal colonies with any degree of certainty.

Smears were made from the glucose broth cultures, stained by Gram's method and examined, and the morphology of different organisms and their frequency noted.

The original plates were again examined after twenty-four hours' and forty-eight hours' incubation, and if any new type of colony was noticed, it was subcultured on to blood agar. The chocolate agar plate was specially examined after 48 hours' incubation for colonies resembling those of haemophilus, and any such colonies were subcultured on fresh plates of the same medium.

Subcultivation was carried out until pure cultures of representatives of all the different colonies on the original plates were obtained. Films of these cultures were then prepared and the morphology and reaction to Gram's stain of the organism was examined. The following modification of Gram's stain recommended by White and Culbertson (1945) gave excellent results and was used throughout:

1. One per cent. solution of crystal violet in distilled water, filtered. The slides were covered with/

this stain and to each slide five drops of a five per cent. solution of sodium bicarbonate were added. The stain was allowed to act for one minute.

2. The stain was then washed off with a solution of one per cent. potassium mercuric iodide in distilled water and the latter allowed to remain for at least one minute.

3. The film was then washed in tap water.

4. Decolorized with acetone (100 per cent.) until no further colour was lost.

5. Washed again in tap water.

6. The film was then counterstained with dilute safranin for one minute (one part of 1 per cent. safranin + 10 parts of distilled water).

7. Finally washed with tap water and dried.

When a large number of slides were being stained, racks were filled in rows and steps 1, 2 and 3 applied to all slides at one time. It was satisfactory to decolorize about six slides at a time, tilting the rack so that the acetone ran off and did not stand on the smears for any appreciable time before washing with tap water. This method gave better differentiation, and it was particularly useful when large numbers of smears of different density were stained at a time, as necessitated by this investigation.

The different organisms isolated from the nose, nasopharynx/

nasopharynx and tonsils of healthy dogs were identified as $N_1, N_2, N_3 \dots NP_1, NP_2, NP_3 \dots T_1, T_2, T_3 \dots$, and the organisms in the same situations from sick dogs were represented as $N_a, N_b, N_c \dots NP_a, NP_b, NP_c \dots T_a, T_b, T_c \dots$ and so on, while the reference numbers of the dogs preceded these initials.

When pure cultures were obtained, such of the organisms as could be definitely identified by means of their colonial form, morphological appearance, and staining reactions, were subjected to special tests for further confirmation and typing, as shown under their respective headings. Organisms which could not be readily identified were subjected to a full series of tests as follows:-

1. Motility: examined for in young rapidly-growing broth cultures 6-8 hours old, incubated at 27°C . and 22°C . respectively.
2. Type of growth in broth.
3. Type of growth on nutrient agar.
4. Presence or absence of haemolysis on 5 per cent. horse blood agar plate.
5. Growth on MacConkey's agar plate.
6. Type of growth in gelatin stab culture and the presence or absence of liquefaction.
7. Löffler's serum - type of growth and the presence or absence of liquefaction.
8. Potato slope - type of growth, particularly the pigmentation.

9. Biochemical reactions. (a) The following series of carbohydrates media were inoculated and incubated at 37°C. and examined daily for acid or acid and gas production, until terminated at the end of fourteen days: glucose, lactose, mannitol, inositol, maltose, dulcitol, sucrose, salicin, raffinose, trehalose, inulin, dextrin, mannose, xylose, sorbitol, arabinose, and glycerol.

(b) Litmus milk: The formation of acid or alkali, clot, clot disrupted by gas, peptonization and other changes were noted for about two weeks.

(c) Methylene blue (1 in 10,000): The ability to grow in the presence of the dye and the production of partial reduction or complete reduction was noted.

(d) Catalase: An agar slope culture was removed after 24 hours' incubation and 1 c.c. of H₂O₂ (10 vol.) was poured over the growth, and the tube was set in an inclined position. Positive reaction was indicated by the production of gas bubbles.

(e) Indole was tested for by taking a five days old culture in peptone water and transferring 2 c.c. of it to a small test tube. 0.5 c.c. of ether was added and the tube shaken thoroughly and then allowed to stand until ether collected on the surface and then 0.5 c.c. of Ehrlich's rosindole reagent was added.

(f) Ammonia: To the remaining peptone water culture in the tube, a few drops of Nessler's reagent were added/

added and the formation of a brown colour was noted as positive and a faint yellow as negative.

(g) Methyl red (M.R.): Tested by adding about two drops of 0.04 per cent. solution of methyl red to about 5 c.c. of glucose phosphate culture after three days' incubation.

(h) Voges-Proskaur reaction: Tested by adding 1 c.c. of a 10 per cent. solution of caustic potash to about 5 c.c. of glucose phosphate culture and allowing it to stand at room temperature for some hours.

(i) Nitrate reduction: Tested on the nitrate broth culture after five days' incubation by adding 1 c.c. of solution A. followed by 1 c.c. of solution B. Positive reaction was indicated by the formation of pink, red or marrow colour.

Solution A. was prepared by adding 22 ml. of distilled water to 1 gm. of A-naphthylamine, dissolved by gentle heat, filtered and 180 ml. of dilute acetic acid (sp. gr. 1.04) added.

Solution B was prepared by dissolving 0.5 gm. of sulphanilic acid in 150 ml. of dilute acetic acid.

(j) Hydrogen sulphide: Brown or black colouration of the lead acetate medium was suggestive of positive reaction (heart extract broth containing 4 per cent. peptone, 2.5 per cent. agar and an equal quantity of one per cent. solution of basic lead acetate).

Organisms which did not grow in ordinary media and which were definitely haemophilic, were grown in haemopeptone water, in order to test their motility and indole production, and their sugar reactions were tested in a special medium recommended by Rivers and Kohn (1921). These media were prepared by the following methods:-

Haemopeptone water:

Peptone (Evan's) = 20 gms.
Sodium chloride = 5 gms.
Distilled water = 1000 c.c.

Boiled and adjusted to pH 7.4

10 c.c. of washed horse red blood cells added and heated to 95°C. Filtered through paper, sterilised through a Mandler filter, tubed in 10 c.c. quantity and incubated for sterility.

Sugar medium for haemophilus:

Peptone (Evan's) = 2 gms.
Sodium chloride = 5 gms.
Shredded agar = 15 gms.
Distilled water = 1000 c.c.

The mixture was boiled and titrated to pH 7.4, filtered and autoclaved in 100 c.c. quantities. After it was taken from the autoclave and while it was still at 95°C. 1 c.c. of washed R.B.C., 10 c.c. of a 10 per cent. solution of the required sugars and enough 25 per cent. alcoholic solution of brom cresol purple, to give a good colour, were added.

While/

While still warm, the medium was tubed and later incubated to test the sterility. While making the medium care was taken to have a good quantity of water of condensation.

... plates, especially the ... plates, ... amount of growth observed on them. ... The growth was ... and the different types of colonies ... that were ... the ... while it was ... in the ...

... which were predominant in the ... were less frequent in the ... and still less in the ... when present in the ... they ... of the total number of colonies, ... in the ... were higher, and in the ... the ... of the colonies.

... were ... in the ... and ... of the colonies in ... of this ... in the cultures of ... in the ... from the ... the ... the ... in the ... in ...

RESULTS

The bacterial flora of the nasal passages differed in several respects from those of the nasopharynx and tonsils. Examination of the primary culture plates, especially the blood agar plates, showed some striking differences regarding the amount of growth observed on them. The growth was less copious and the different types of colonies were fewer in number on the culture plate from the nose than from that of the nasopharynx, while it was highest in the tonsil culture.

Streptococci, which were predominant in the nose, were less frequent in the nasopharynx and still less in the tonsils. When present in the tonsils, they comprised only a small percentage of the total number of colonies, whereas in the nasopharynx their numbers were higher, and in the nose they constituted the majority of the colonies.

Similarly, haemolytic streptococci were more frequent in the tonsils than in the nasopharynx and comprised only a small percentage of the colonies in the nasal swabs. The number of colonies of this organism was very large in the cultures of tonsil swabs, somewhat less in those of the nasopharynx, but only a few, when present, in cultures from the nose. It was also observed that the growth was more copious in the cultures of the swabs from sick dogs than in those from corresponding situations in normal dogs.

No marked differences were observed regarding the numbers and types of colonies in the cultures obtained from living or destroyed dogs. Similarly, cultures of half a dozen excised tonsils did not show any marked difference when compared with the swabs taken from the same dogs before and after death.

The following organisms were isolated from the normal and sick dogs, and the appropriate study of each species was made as dealt with under their respective headings:-

1. Staphylococci.
2. Beta-haemolytic streptococci.
3. Non-haemolytic streptococci
4. Gram-negative cocci (Neisseria).
5. Haemophilus bronchisepticus.
6. Coliform bacilli.
7. Diphtheroid bacilli.
8. Anthracoides (Bacillus subtilis group).
9. Bacterium alkaligenes.
10. Bacillus Friedländer.
11. Haemophilus canis.
12. Proteus sp.
13. Leptotrichia sp.
14. Unidentified.

The absolute and relative frequencies of these organisms in the nose, nasopharynx and tonsils of normal and sick dogs are shown in Tables IIIa and IIIb respectively, and their summaries in Tables III and IV.

TABLE III

Summary of the incidence of different organisms in the normal and sick dogs.

Organisms	Normal dogs (75)		Sick dogs (25)		Difference per cent.
	No. positive	Per cent. positive	No. positive	Per cent. positive	
Staphylococci	53	70.7	21	84	+ 13.3
Beta haemolytic streptococci	48	64	17	68	+ 4
Non-haemolytic streptococci	58	77.3	24	96	+ 18.7
Gram-negative cocci	45	60	14	56	- 4
H. bronchisepticus	14	18.7	13	52	+ 33.3
Coliform bacilli	16	21.3	12	48	+ 26.7
Diphtheroid bacilli	14	18.7	7	28	+ 9.3
B. subtilis group	12	16	5	20	+ 4
Bacterium alkaligenes	27	36	15	60	+ 24
Bacillus of Friedlander	-	-	4	16	+ 16
Haemophilus canis	9	12	2	8	- 4
Proteus sp.	12	16	7	28	+ 12
Leptotrichia sp.	18	24	7	28	+ 4
Unidentified group (a)	45	60	16	64	+ 4
Unidentified group (b)	36	48	11	44	- 4
Unidentified group (c)	27	36	10	40	+ 4

TABLE IV

Summary of the incidence of different organisms in the nose, nasopharynx and tonsils of normal and sick dogs.

Class	Situation	Staphylococci	Beta haemolytic streptococci	Non-haemolytic streptococci	Gram-negative cocci	Haemophilus bronchisepticus	Coliform bacilli	Diphtheroid bacilli	B. subtilis group	Bacterium alkaligenes	Bacillus of Friedlander	Haemophilus canis	Proteus sp.	Leptotrichia sp.	Unidentified		
															Group (a) %	Group (b) %	Group (c) %
Normal	N	66.7	13.3	28	38.7	14.7	14.7	14.7	16	21.3	-	25.3	6.7	-	8	5.3	1.3
	NP	34	48	46	48	10	4	6	-	6	-	6	4	18	58	32	30
	T	24	62.7	65.3	26.7	4	5.3	2.7	-	20	20	-	4	6.7	24	60	37.3
Sick	N	84	32	48	32	52	20	16	20	40	8	8	20	-	8	8	8
	NP	58.8	47	52.9	35.3	41.2	17.6	11.8	-	35.3	6	5.9	11.8	23.5	5.9	35.3	29.4
	T	36	68	76	32	20	28	4	4	32	8	4	16	24	64	36	36

STAPHYLOCOCCI

Preface. Much work has been devoted to the study of staphylococci obtained from human sources in order to distinguish pathogenic from non-pathogenic strains. Various properties, such as pigment production, fermentation of mannitol, liquefaction of gelatin, the production of haemolysis on blood agar, the production of filterable toxins and their differentiation by haemolytic, skin-necrotising and lethal effects, have all been examined. Other properties, such as the demonstration of leucocidin, fibrinolysin, and the lethal effect of the staphylococci themselves on experimental animals, have also been studied.

Recent work on the classification of staphylococci, particularly by Chapman et al (1934), Cruickshank (1937), Fairbrother (1940), and Christie and Keogh (1940), has shown that while some of the other tests are of considerable value, the only absolute criterion of pathogenicity is the production of coagulase. Some workers now accept the division of the genus *Staphylococcus* into *Staphylococcus pyogenes* (coagulase-positive strain), and *Staphylococcus saprophitiosus* (coagulase-negative strain), as suggested by Fairbrother (1940).

A search of the literature revealed that until recently very little of the work done on staphylococci of/

of animal origin had been directed to discovering criteria of pathogenicity. Dumas (1914) examined the cultural and biochemical reactions of seventeen strains, eight of which were of animal origin and nine of human. Minett (1936) examined fifty-one pathogenic strains from various animal species, with particular reference to their toxins, but did not determine their capacity to form coagulase. He concluded that the production of beta-toxin was a characteristic feature of haemolytic staphylococci obtained from animals, and that strains from dogs could be distinguished by their greater proteolytic power. Cruickshank (1937) examined six animal strains of unspecified origin for coagulase production by the tube test. All were coagulase-positive. Bell (1940) studied sixty-one strains of diverse animal origin, including ten strains from dogs, for toxin formation, pigment production, haemolysis production, coagulase production and fermentation of mannitol, and concluded that coagulase production more nearly correlated with haemolysin production than did fermentation of mannitol. Field and Smith (1945) examined a large number of strains of staphylococci of human and animal origin and concluded that coagulase production was confined to pathogenic staphylococci. Christie, North and Parkin (1946) have reported the examination of 1,027 strains of staphylococci/

staphylococci from human and animal sources, all of which were tested for coagulase production, haemolysis on sheep's blood agar, formation of pigment and fibrinolysin and fermentation of mannitol. A number of them were also examined for pathogenicity to mice. These authors concluded that a strain may produce coagulase and still be non-pathogenic, and that all pathogenic strains produce haemolysis on sheep's blood agar, while non-pathogenic strains do not. Recently, Smith (1947), in his more comprehensive study of staphylococci of animal origin, with particular regard to the determination of criteria of pathogenicity, examined 173 strains of diverse animal origin, including 39 from dogs, for various properties, including dermatoin production, the lethal effect of toxin and organisms on mice and rabbits, and concluded that the production of coagulase was the only absolute criterion of pathogenicity. Pathogenic strains also showed liquefaction of solid serum and fibrin, the production of beta-haemolysis on sheep's blood agar, and of haemotoxin, dermatoin, and lethal toxin, but these properties were shown only by some of them. According to this investigation, the pathogenic strains from dogs formed a fairly distinct group in that they nearly all produced white pigment, formed much beta-toxin but little or no alphanotoxin, were non-lethal to mice and rabbits; most of them actively liquefied solid serum and all produced fibrinolysin.

Methods followed in this survey

All the strains isolated from both normal and sick dogs were examined for pigment production, fermentation of lactose and mannitol, liquefaction of solid serum, coagulase production and haemolysis on blood agar in the manner described below.

Pigment production. The chromogenic characters were compared on three different types of media, viz., nutrient agar, Löffler's serum, and 33 per cent. milk agar (Christie and Keogh, 1940).

Liquefaction of solid serum. Horse serum was used for this purpose. Incubation was at 37°C. for ten days.

Gelatin liquefaction. In stab culture incubated at 22°C. and observations made for two weeks.

Coagulase production. The method advocated by Topley & Wilson (1946 a) was followed. 0.1 ml. of an overnight broth culture was mixed with 1 c.c. of a freshly prepared 1/10 dilution of rabbit plasma in saline. The mixture was incubated at 37°C. for thirty-six hours, and if no clot had formed by that time, it was left overnight at room temperature and re-examined. Two control tubes, one containing diluted plasma alone and the other inoculated with a known coagulase-positive strain, were always put up with this test.

Haemolysis on blood agar. The method adopted by Smith (1947) was employed. Broth cultures of the strains/

strains were spot inoculated on to a 10 per cent. sheep's blood agar and examined after twenty-four hours' incubation at 37°C. and then left at room temperature for a further period of four days.

Results.

Staphylococci were isolated from fifty-three (70.7 per cent.) of the normal dogs and from twenty-one (84 per cent) of the sick dogs, from one or more situations.

Thirty-seven (49.3 per cent.) of the normal and seventeen (68 per cent.) of the sick dogs yielded pathogenic (coagulase-positive) strains. Their frequency of occurrence in the nose, nasopharynx and tonsils of normal and sick dogs are represented in Table VI. From normal dogs, 66.7 per cent. of the nose, 34 per cent. of the nasopharynx and 24 per cent. of the tonsil swabs were positive, while the frequency increased to 84 per cent., 58.8 per cent. and 36 per cent. respectively in the corresponding swabs from sick dogs.

Pigment production. Pigment production was found to vary according to the medium, temperature and duration of incubation employed. But the best results were obtained with 33 per cent. milk agar, readings being taken after two days at 37°C. followed by four days at room temperature.

Of one hundred and twenty-five strains, only sixteen (12.8 per cent.) produced golden pigment, while/



TABLE V

Percentage carrier rates for Staphylococci in relation to Coagulase-positive strains

Class	No. of dogs swabbed	Number Positive	Percent Positive	No. positive for pathogenic (coagulase +) strains	Percent positive for pathogenic strains.
Normal	75	53	70.7	37	49.3
Sick	25	21	84	17	68
Combined	100	74	74	54	54

TABLE VI

The frequency of Staphylococci in the nose, nasopharynx and tonsils of normal and sick dogs.

Situation	Normal Dogs			Sick Dogs			Combined		
	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%
Nose	75	50	66.7	25	21	84	100	71	71
Nasopharynx	50	17	34	17	10	58.8	67	27	40.3
Tonsils	75	18	24	25	9	36	100	27	27

while the rest (87.2 per cent.) were white. Of the eighty-three coagulase-positive strains, only six (7.2 per cent.) were aureus and among forty-two coagulase-negative strains, ten (23.8 per cent.) were aureus. This is in agreement with the observations made by Smith (1947) that only a small percentage of pathogenic staphylococci from dogs form aureus pigment, while the rest produce white pigment, and that a fair percentage of non-pathogenic strains also produce aureus pigment.

Coagulase production. Of eighty-five strains from normal dogs, fifty-one (60 per cent.) and of forty strains from sick dogs, thirty-two (80 per cent.) were positive for coagulase. On the whole, of 125 strains from normal and sick dogs, eighty-three (66.4 per cent.) formed coagulase. Twenty-one (25.3 per cent.) of the coagulase-positive strains produced firm solid coagulum with rabbit plasma within three hours' incubation. Forty-five (54.2 per cent.) of them did so in six hours and seven (20.5 per cent.) were found positive only after leaving the tubes overnight.

Liquefaction of solid serum. Forty-seven (37.6 per cent.) of the total of 125 strains were able to liquefy solid serum. The majority of them acted rapidly and copiously, causing deep depressions in the medium within twenty-four hours, which increased considerably on further incubation. Of the eighty-three/

three coagulase-positive strains, forty-three (51.8 per cent.) liquefied serum. Only four (9.5 per cent.) of the coagulase-negative strains liquefied this medium. The process in this case was slow. Such proteolytic power was attributed to the pathogenic strains of staphylococci from dogs by Minett (1936) and Smith (1947), but none of the non-pathogenic strains studied by Smith were able to liquefy either dog, horse, or ox serum.

All the coagulase-positive strains which liquefied serum produced either alpha-beta type of haemolysis or the beta type alone, but the four coagulase-negative strains, namely 37N₁, 52N₂, 37T₂, and 59 N₁ which had feeble proteolytic power failed to produce either beta- or alpha-beta type of haemolysis. Two of them produced alpha-type only, while the other two were inert on sheep's blood agar.

Liquefaction of gelatin. Of the total of 125 strains, one hundred and eleven (88.8 per cent.) liquefied gelatin. All the coagulase-positive strains and 66.7 per cent. of the coagulase-negative strains liquefied this medium, although the degree of liquefaction varied with different cultures. Even the rapid and extensive liquefaction did not necessarily correlate with coagulase production. This test again is of no practical value in differentiating pathogenic and non-pathogenic strains from dogs. This is also quite in agreement with the observations made by Smith (1947).

Summary of the characteristics of Staphylococci isolated from normal dogs.

Situation	Number of strains isolated	Number Positive for Coagulase	Number liquefying horse serum	Number liquefying gelatin	Number fermenting mannitol	Number showing haemolysis on sheep blood agar			
						Alpha type only	Beta type only	Alpha + Beta type	Total
Nose	50	29	18	45	37	6	2	21	29
Nasopharynx	17	11	7	12	9	-	1	9	10
Tonsils	18	11	8	15	13	1	2	9	12
Total	85	51	33	72	59	7	5	39	51

60% produced Coagulase

38.8% liquefied solid serum

84.7% liquefied gelatin

69.4% fermented mannitol.

60% produced one or the other type of haemolysis on sheep blood agar.

Summary of the characteristics of Staphylococci isolated from sick dogs.

Situation	Number of strains isolated	Number Positive for Coagulase	Number Liquefying horse serum	Number Liquefying gelatin	Number fermenting mannitol	Number showing haemolysis on sheep blood agar			
						Alpha type only	Beta type only	Alpha + Beta type	Total
Nose	21	15	6	20	15	1	1	14	16
Nasopharynx	10	9	3	10	10	1	-	9	10
Tonsils	9	8	5	9	8	-	1	7	8
Total	40	32	14	39	33	2	2	30	34

80% produced coagulase.

35% liquefied solid serum.

97.5% liquefied gelatin.

82.5% fermented mannitol.

85% produced one or the other type of haemolysis on sheep blood agar.

Summary of the characteristics of Staphylococci isolated from normal and sick dogs (combined)

Situation	Number of strains isolated	Number Positive for Coagulase	Number liquefying horse serum	Number liquefying gelatin	Number fermenting mannitol	Number showing haemolysis on sheep blood agar			
						Alpha type only	Beta type only	Alpha + beta type	Total
Nose	71	44	24	65	52	7	3	35	45
Nasopharynx	27	20	10	22	19	1	1	18	20
Tonsils	27	19	13	24	21	1	3	16	20
Total	125	83	47	111	92	9	7	69	85

66.4% produced Coagulase
 37.6% liquefied solid serum
 88.8% liquefied gelatin
 73.6% fermented mannitol.
 68% produced one or the other type of haemolysis on sheep blood agar.

Fermentation reaction. Of eighty-five strains from normal dogs, fifty-nine (69.4 per cent.) and of forty strains from sick dogs, thirty-three (82.5 per cent.) fermented mannitol. All but four coagulase-positive strains and twenty (47.6 per cent.) coagulase-negative strains fermented mannitol. The majority of the strains showed slow action on this sugar, usually requiring three to five days for definite reaction. Lactose was fermented by all the strains with the exception of one (strain 20NP_c), which did not ferment either lactose or mannitol, but was coagulase-positive and produced alpha-beta type of haemolysis on sheep's blood agar. The results indicate, therefore, that lactose and mannitol fermentation do not serve to classify the pathogenic strains, or to distinguish them from non-pathogenic ones. This agrees with the findings of Cowan (1939), who stated that greater correlation between mannitol fermentation and coagulase production is found among strains of human origin than among those of animal origin. Bell (1940) and Smith (1947) also drew the same conclusion.

Haemolysis on sheep's blood agar. For the purpose of classification only, a clear zone of haemolysis is referred to as alpha type, a semi-clear zone as beta type, and a clear zone surrounded by a semi-clear zone as alpha-beta type.

Fifty-one (60 per cent.) of the strains from normal/

TABLE X

Comparison between Coagulase-positive and Coagulase-negative Strains

Characteristics	83 Coagulase-positive Strains			42 Coagulase-negative Strains		
	Positive	Negative (albus)	Positive per cent	Positive	Negative (albus)	Positive per cent.
Aureus Pigmentation	6	77 (albus)	7.2	10	32 (albus)	23.8
Serum liquefaction	43	40	51.8	4 (slow)	38	9.5
Gelatin liquefaction	83	-	100	28	14	66.7
Lactose fermentation	82	1	98.8	42	-	100
Mannitol fermentation	79	4	95.2	20	22	47.6
Haemolysis on sheep blood agar	78 A type only 2 B " " 7 A+B type 69	5	93.9	7 A type only	35	16.7 A type only

A = alpha.

B = beta.

A+B = alpha + beta.

normal dogs and thirty-four (85 per cent.) from sick dogs produced haemolysis. Of the eighty-three coagulase-positive strains, seventy-eight (93.9 per cent.) showed haemolysis, two of alpha type only, seven of beta type only, and sixty-nine alpha-beta type. Only five were inert. Among coagulase-negative strains, seven produced alpha type only, the remaining thirty-five were completely inert. None of the coagulase-negative strains produced either beta type alone or alpha-beta type. Smith (1947) also did not find any of his twelve non-pathogenic (coagulase-negative) strains producing alpha-beta or beta type of haemolysis, though three of them produced alpha type only. All the pathogenic (coagulase-positive) strains of Smith produced haemolysis, unlike the exceptions noted in this study.

No.	Strain	Coagulase	Haemolysis	Other
1	Alpha	+	+	
2	Alpha	+	+	
3	Alpha	+	+	
4	Alpha	+	+	
5	Alpha	+	+	
6	Alpha	+	+	
7	Alpha	+	+	
8	Alpha	+	+	
9	Alpha	+	+	
10	Alpha	+	+	
11	Alpha	+	+	
12	Alpha	+	+	
13	Alpha	+	+	
14	Alpha	+	+	
15	Alpha	+	+	
16	Alpha	+	+	
17	Alpha	+	+	
18	Alpha	+	+	
19	Alpha	+	+	
20	Alpha	+	+	
21	Alpha	+	+	
22	Alpha	+	+	
23	Alpha	+	+	
24	Alpha	+	+	
25	Alpha	+	+	
26	Alpha	+	+	
27	Alpha	+	+	
28	Alpha	+	+	
29	Alpha	+	+	
30	Alpha	+	+	
31	Alpha	+	+	
32	Alpha	+	+	
33	Alpha	+	+	
34	Alpha	+	+	
35	Alpha	+	+	
36	Alpha	+	+	
37	Alpha	+	+	
38	Alpha	+	+	
39	Alpha	+	+	
40	Alpha	+	+	
41	Alpha	+	+	
42	Alpha	+	+	
43	Alpha	+	+	
44	Alpha	+	+	
45	Alpha	+	+	
46	Alpha	+	+	
47	Alpha	+	+	
48	Alpha	+	+	
49	Alpha	+	+	
50	Alpha	+	+	
51	Alpha	+	+	
52	Alpha	+	+	
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65	Alpha	+	+	
66	Alpha	+	+	
67	Alpha	+	+	
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69	Alpha	+	+	
70	Alpha	+	+	
71	Alpha	+	+	
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88	Alpha	+	+	
89	Alpha	+	+	
90	Alpha	+	+	
91	Alpha	+	+	
92	Alpha	+	+	
93	Alpha	+	+	
94	Alpha	+	+	
95	Alpha	+	+	
96	Alpha	+	+	
97	Alpha	+	+	
98	Alpha	+	+	
99	Alpha	+	+	
100	Alpha	+	+	

TABLE XI

Characteristics of Staphylococci

S E R I E S	Pigmentation	Biochemical		Coagulase (Rabbit Plasma)	Liquefaction of Gelatin	Liquefaction of Solid Serum	Type of haemolysis on Sheep Blood Agar
		Lactose	Mannitol				
		1 N1	Albus				
1 NP2	Albus	A	-	-	-	-	-
2 N2	Albus	A	A	+++	+	+	A+B (alpha - beta)
2 NP1	Albus	A	A	+++	+	+	A+B
3 N4	Albus	A	A	++	+	+	A+B
4 N1	Albus	A	-	-	+	-	-
4 T4	Albus	A	-	-	+	-	-
4 NP1	Albus	A	-	-	+	-	-
5 N1	Albus	A	-	-	-	-	-
7 N2	Albus	A	A	++	+	+	A+B
7 NP1	Albus	A	A	++	+	+	A+B
8 N1	Albus	A	A	+	+	-	A+B
8 NP1	Albus	A	A	+	+	-	A+B
8 N1	Albus	A	-	-	-	-	-
10 N1	Albus	A	-	-	-	-	-
10 NP1	Albus	A	-	-	-	-	-
12 N1	Albus	A	A	+++	+	+	A+B
14 N1	Albus	A	A	++	+	-	A+B
14 T1	Albus	A	A	++	+	-	A+B
15 N2	Albus	A	A	++	+	+	A+B
19 N1	Albus	A	-	-	+	+	A+B
19 NP3	Albus	A	-	-	+	-	-
20 N3	Albus	A	A	++	+	-	A+B
21 N1	Albus	A	A	++	+	+	A+B
22 N1	Aureus	A	A	+	+	+	-
22 T5	Albus	A	A	-	+	-	-
22 NP3	Albus	A	A	++	+	-	-
24 N2	Albus	A	A	++	+	+	A+B
24 T4	Albus	A	A	++	+	+	A+B
25 N1	Albus	A	A	++	+	+	-
27 N1	Albus	A	-	-	+	-	-
28 N21	Albus	A	A	-	+	-	A+B
28 NP1	Albus	A	A	+++	+	+	A+B
31 N1	Albus	A	-	-	+	-	-
35 N1	Albus	A	A	-	+	-	A only
36 N1	Aureus	A	A	++	+	-	A+B
37 N1	Albus	A	A	-	+	+	A only
37 T2	Albus	A	A	-	+	+	-
37 NP1	Aureus	A	A	+	+	+	B only
40 T1	Albus	A	-	-	+	-	-
41 N1	Aureus	A	A	-	+	-	-

TABLE XI Contd.

S E R I E S	Pigmentation	Biochemical		Coagulase (Rabbit Plasma)	Liquefaction of Gelatin	Liquefaction of Solid Serum	Type of Haemolysis on Sheep Blood Agar
		Lactose	Mannitol				
		Biochemical					
43 N1	Albus	A	A	++	+	+	A+B
43 NP2	Albus	A	A	+++	+	+	A+B
44 N1	Albus	A	A	++	+	-	B only
47 T3	Albus	A	-	-	-	-	-
48 N1	Albus	A	A	+++	+	+	A+B
49 N1	Albus	A	-	+	+	-	A+B
50 N1	Albus	A	A	++	+	+	B only
50 NP1	Albus	A	-	+	+	-	A+B
50 T1	Albus	A	A	+	+	-	A+B
51 N1	Aureus	A	A	-	+	-	A only
51 T1	Albus	A	A	+++	+	+	A+B
52 N2	Albus	A	A	-	+	+	A only
52 NP1	Albus	A	-	++	+	+	A+B
52 T2	Albus	A	-	++	+	+	A+B
53 N1	Albus	A	A	+++	+	-	-
54 N1	Albus	A	A	+++	+	-	-
55 N1	Aureus	A	A	+++	+	-	A only
55 NP3	Albus	A	A	++	+	-	A+B
56 N2	Aureus	A	A	-	+	-	-
56 T1	Albus	A	A	+++	+	-	A+B
57 N3	Aureus	A	A	-	+	-	-
58 N1	Albus	A	-	++	+	-	A+B
58 T1	Albus	A	-	+++	+	+	B only
59 T1	Albus	A	A	+++	+	+	A+B
59 N1	Albus	A	-	-	+	+	-
60 N1	Albus	A	-	-	-	-	-
60 T1	Albus	A	A	+++	+	-	A+B
63 N2	Albus	A	A	-	+	-	-
63 NP1	Albus	A	A	-	-	-	-
64 N1	Albus	A	A	+++	+	-	-
66 N1	Aureus	A	A	-	+	-	-
66 T3	Albus	A	A	+++	+	+	A+B
67 N1	Aureus	A	A	-	+	-	-
68 N1	Aureus	A	A	-	+	-	-
69 N1	Albus	A	-	++	+	+	A+B
69 NP1	Albus	A	-	++	+	+	A+B
70 N1	Albus	A	A	-	+	-	-
70 T6	Albus	A	A	-	-	-	-
71 T2	Aureus	A	A	+++	+	-	A only
73 N1	Albus	A	A	++	+	+	A+B
73 T1	Albus	A	A	++	+	+	B only
75 N1	Albus	A	-	++	+	+	A+B

HAEMOLYTIC STREPTOCOCCI

Methods of identification.

Single colonies showing beta-haemolysis on the primary blood agar plate were subcultured to fresh blood agar plates and after eighteen hours' incubation at 37°C. the plates were examined for haemolysis and a film was made, stained, and examined. If found to be small cocci in chains, it was recorded as haemolytic streptococci. A test for soluble haemolysin was not performed. In every case a colony picked up from the primary plate, presumed to be of haemolytic streptococci by its characteristic disc-like appearance with a clear zone of haemolysis, definitely proved to be so when subcultured and examined.

All strains were identified by Lancefield's precipitation method to ascertain the distribution of various groups in the nose, nasopharynx and tonsils of both normal and sick dogs.

The acid extraction method of Lancefield (1933) was employed for the purpose of grouping. The organism was grown in 100 ml. of glucose broth and, after centrifugation of the culture, the deposit was extracted with 4 ml. N/20 HCl in saline by boiling for ten minutes in a water bath. After cooling and adding two drops of phenol red indicator, the extract/

extract was neutralized with N/1 NaOH solution, centrifuged and the clear extract was tested against the precipitating sera. All the sera, excepting that of group M, were obtained from Messrs Burroughs, Wellcome & Co. Serum of group M was not available from any of the commercial laboratories but was kindly supplied by Dr R. M. Fry, of Cambridge.

All the strains of haemolytic streptococci isolated in this investigation, without a single exception, fell into one or other of Lancefield's groups, and the acid extraction method was found perfectly satisfactory for all of them. All the strains which showed group G reaction were tested for cross-precipitation with group C serum, and, similarly, group C strains were tested with group G serum. But in no case was cross-precipitation observed. It may, however, be pointed out that all the strains were grouped when freshly isolated. The various groups were tested for their fermentative ability as complementary tests for their identification: group G in Aesculin, lactose, raffinose, glycerol, litmus milk, and for the hydrolysis of sodium hippurate; group C in lactose, trehalose and sorbitol; groups L and M in lactose, trehalose, sorbitol, mannitol, salicin and litmus milk; group A in lactose, sucrose, trehalose, sorbitol, salicin, raffinose, mannitol, inulin, litmus milk, and for the hydrolysis of sodium hippurate; and group F in aesculin, trehalose, sorbitol, lactose, salicin and litmus milk.

Result.

The percentage of carriers of haemolytic streptococci observed in this investigation was not so high as that recorded by Pilot et al (loc. cit.), which reached up to 92 per cent. in young dogs, but it compared favourably with the result of one of the surveys of Garside (loc. cit.) in which the positive carrier rate was recorded as 60 per cent. among the general dog population of Edinburgh city.

The difference in the carrier rate of streptococci in the two groups of dogs, normal (64 per cent.) and sick (68 per cent.) is not very significant. For all practical purposes, the combined result (65 per cent.) may be taken as the normal figure,

A striking difference in the incidence of this organism in the nose, nasopharynx and tonsils was observed: while the positive carrier rate in the nose was 13.3 per cent., it increased in the nasopharynx to 48 per cent., whereas it was as high as 62.7 per cent. in the tonsils. Here again, the difference in two groups of dogs was not very marked, except in the nose, where it increased from 13.3 per cent. in the normal to 32 per cent. in the sick. In all positive cases, this organism was present in the tonsils, excepting in one dog, where it was found only in the nasopharynx. There was not a single other instance where it was present in the nose or nasopharynx and absent in the tonsils.

TABLE XII

Percentage Carrier Rates for Haemolytic Streptococci.

Group	Number of dogs swabbed	Number Positive	Percent Positive
Normal	75	48	64
Sick	25	17	68
Combined	100	65	65

TABLE XIII

The Frequency of Haemolytic Streptococci in the Nose, Nasopharynx and Tonsils.

Situation	Normal Dogs			Sick Dogs			Combined		
	Number swabbed	Number Positive	Percent Positive	Number Swabbed	Number Positive	Percent Positive	Number Swabbed	Number Positive	Percent Positive
Nose	175	10	13.3	25	8	32	100	18	18
Nasopharynx	50	24	48	17	8	47	67	32	47.7
Tonsils	75	47	62.7	25	17	68	100	64	64

One hundred and fourteen strains of this organism thus isolated were distributed amongst six serological groups of Lancefield, the most predominant of them being group G, to which 48.2 per cent. of the strains belonged. The next in frequency was group M, which totalled 23.7 per cent. Groups C and L were 13.2 per cent. and 10.5 per cent. respectively. Group A, which formed 2.6 per cent. of the total strains, was found present in the nose and tonsils of only one normal dog and in the tonsils of one sick dog. Similarly, group F, which formed 1.8 per cent. of the strains, was present in the tonsils of one and in the nasopharynx of another normal dog.

In one dog, group A strain was isolated from the tonsils, whereas the nose of the same dog yielded group G strain. Similarly, in another dog, group C was isolated from the nose, while the nasopharynx and tonsils were positive for group G. Excepting these two instances, in all the rest of the dogs the strains belonging to the same group were recovered from more than one situation whenever it was present.

On the whole, 28 per cent. of the normal and 36 per cent. of the sick dogs harboured group G, while group M decreased from 20 per cent. in the normal to 12 per cent. in the sick. Group G was isolated from 8 per cent. of the normal and 12 per cent./

TABLE XIII

Number of Strains and Groups of Haemolytic Streptococci isolated from Nose, Nasopharynx and Tonsils of Normal Dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total Strains
Nose	9	4	2	2	1	-	18
Nasopharynx	17	3	7	4	-	1	32
Tonsils	29	8	18	6	2	1	64
Grand Total	55	15	27	12	3	2	114

TABLE XIV

Number of Strains and Groups of Haemolytic Streptococci isolated from Nose, Nasopharynx and Tonsils of Normal and Sick Dogs

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total Strains
Nose	9	4	2	2	1	-	18
Nasopharynx	17	3	7	4	-	1	32
Tonsils	29	8	18	6	2	1	64
Grand Total	55	15	27	12	3	2	114

48.2% Group G

13.2% " C

23.7% " M

10.5% " L

2.6% " A

1.8% " F

48.2% Group G
13.2% " C
23.7% " M

10.5% Group L
2.6% " A
1.8% " F

TABLE XV

Number of Strains and Groups of Haemolytic Streptococci isolated from Nose, Nasopharynx and Tonsils of normal dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total strains
Nose	5	2	2	-	1	-	10
Nasopharynx	11	3	6	3	-	1	24
Tonsils	21	5	15	4	1	1	47
Total	37	10	23	7	2	2	84

45.7% Group G
12.3% " C
28.4% " M

8.6% Group L
2.5% " A
2.5% " F

TABLE XVI

Number of Strains and Groups of Haemolytic Streptococci isolated from Nose, Nasopharynx and Tonsils of sick dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total strains
Nose	4	2	-	2	-	-	8
Nasopharynx	6	-	1	1	-	-	8
Tonsils	8	3	3	2	1	-	17
Total	18	5	4	5	1	-	33

54.5% Group G
15.2% " C
12.1% " M

15.2% Group L
3.0% " A
0.0% " F

TABLE XVII

Incidence of different groups of Haemolytic Streptococci in normal and sick dogs.

Class	Group G		Group M		Group C		Group L		Group F		Group A	
	Number Positive	Percent Positive	Number Positive	Percent Positive	Number Positive	Percent Positive	Number Positive	Percent Positive	Number Positive	Percent Positive	Number Positive	Percent Positive
Normal (75)	21	28	15	20	6	8	4	5.3	2	2.7	1	1.3
Sick (25)	9	36	3	12	3	12	2	8	-	-	1	4
Combined (100)	30	30	18	18	9	9	6	6	2	2	2	2

cent. of the sick; similarly, 5.3 per cent. of the normal and 8 per cent. of the sick showed group L: group F was recovered from only 2 per cent. of the normal and none at all from the sick; group A too was found in 1.3 per cent. of the normal and 4 per cent. of the sick dogs.

The biochemical reactions of the 114 strains are set out in detail under their respective groups in Tables XVIII, XIX, XX, XXI, XXII, XXIII. None of the group G strains hydrolysed sodium hippurate. All, without exception, fermented aesculin. Lactose was fermented by all strains except five. Only three strains fermented raffinose, and five strains failed to clot milk.

These reactions are in agreement with the reactions of the three types described by Smith and Sherman (1938), but the fermentation of glycerol is an additional factor. Glycerol is not fermented by any one of the three types described by these authors, but the possible explanation is that their classification was based on the strains of human origin, whereas the same authors observed that group G of animal origin had the property of fermenting this sugar. Forty-three out of the fifty-five strains fermented glycerol. Action on this sugar was rather slow and invariably it took three to five days to bring about the complete reaction. Five strains which/

which did not clot milk and fermented only aesculin may be classified as "pyogenes-like" G's.

Group M strains, which came next to G in number, were found more frequently in normal dogs than in sick dogs. Growth was extremely poor in glucose broth, and often serum had to be added to the medium to obtain sufficient growth for the purpose of performing the precipitation test. On blood agar, the colonies were small with a wide zone of beta-haemolysis. The biochemical reactions were rather constant. All the strains, with the exception of two, fermented lactose but not trehalose, mannitol, sorbitol or salicin. This reaction is in agreement with the findings of Fry (1941). But the two exceptions recorded fermented sorbitol in addition to lactose. All the strains produced slight acid in litmus milk, with the exception of two, which had no action. None clotted milk.

Group C formed the third group in numbers. From the reactions in lactose, sorbitol and trehalose, it was clear that the three types mentioned by Bazeley and Battle (1940) were encountered. Of the fifteen strains, five fermented lactose and sorbitol (Type 2), four fermented trehalose (Type 4), six fermented trehalose and lactose (Type 5). Types 1 and 3 were not met with. It should, however, be noted that the strains studied by Bazeley and Battle were all from horses. Five strains fermenting lactose/
lactose/

lactose and sorbitol belonged to the "animal pyogenes" group, while the remaining ten fermenting lactose and trehalose, or trehalose alone, belonged to the "human C" group (Sherman, 1937).

Colonies of group L, when first isolated, were very small with a wide zone of haemolysis. Unlike group M, these strains grew well in glucose broth.

Lactose and trehalose were fermented by all the twelve strains, while mannitol and sorbitol were not acted upon by any of them.

Contrary to the findings of Fry (1941), the reaction in salicin was variable: eight strains fermented this sugar, while four did not. It should, however, be mentioned that the action on this sugar by some of the strains was rather slow (2-4 days).

The remaining two groups, A and F, were very sparse in number. Three strains of group A, isolated from one normal and one sick dog, showed the typical reactions of streptococcus pyogenes.

Two strains of group F were isolated from two normal dogs - one from the tonsils and the other from the nasopharynx. They grew very slowly on blood agar plates, forming minute pinpoint, transparent colonies surrounded by a narrow zone of beta-haemolysis. Both the strains fermented aesculin, lactose and salicin, but not trehalose and sorbitol. Both curdled milk.

TABLE XVIII

Biochemical ReactionsGROUP G.

Strain	Sodium Hiippurate	Aesculin	Lactose	Raffinose	Glycerol	Litmus Milk
4 T1	-	+	-	-	-	A
4 NP1	-	+	-	-	-	A
5 T1	-	+	+	-	-	Ac
5 NP1	-	+	+	-	-	Ac
6 N2	-	+	-	-	-	A
6 T1	-	+	-	-	-	A
6 NP3	-	+	-	-	-	A
7 T11	-	+	+	-	+	ACR
7 NP3	-	+	+	-	+	ACR
11 NP1	-	+	+	-	+	AC
11 T1	-	+	+	-	+	AC
14 NP4	-	+	+	-	-	AC
14 T6	-	+	+	-	-	AC
16 T1	-	+	+	-	+	AC
21 T1	-	+	+	+	+	ACR
24 NP2	-	+	+	-	+	AC
24 T2	-	+	+	-	+	AC
27 T1	-	+	+	-	-	A
28 N1	-	+	+	-	+	AC
28 T1	-	+	+	-	+	AC
25 NP3	-	+	+	-	+	AC
35 T1	-	+	+	-	+	AC
41 NP3	-	+	+	-	+	ACR
41 T3	-	+	+	-	+	ACR
43 T1	-	+	+	+	+	AC
43 NP1	-	+	+	+	+	AC
52 N11	-	+	+	-	+	ACR
52 T1	-	+	+	-	+	ACR
54 T5	-	+	+	-	+	ACR
56 N4	-	+	+	-	+	ACR
56 T7	-	+	+	-	+	ACR
59 T2	-	+	+	-	+	ACR
60 T2	-	+	+	-	+	ACR
69 T3	-	+	+	-	+	ACR
73 N2	-	+	+	-	-	AC
73 T2	-	+	+	-	-	AC

TABLE XVIII Contd.

Biochemical Reactions.

Strain	Sodium H ippurate	Aesculin	Lactose	Raffinose	Glycerol	Litmus Milk.
1 NPa	+	-	-	-	+	AC
1 Tf	-	+	-	-	+	AC
4 Td	-	+	+	-	+	ACR
4 NPd	-	+	+	-	+	ACR
6 Nb	-	+	+	-	-	AC
6 Te	-	+	+	-	-	AC
6 NPe	-	+	+	-	-	AC
8 Na	-	+	+	-	+	AC
8 Tg	-	+	+	-	+	AC
12 Na	-	+	+	-	+	ACR
17 Na	-	+	+	-	+	AC
17 Ta	-	+	+	-	+	AC
20 Te	-	+	+	+	+	ACR
20 NPe	-	+	+	+	+	ACR
24 NPa	-	+	+	-	+	ACR
24 Ta	-	+	+	-	+	ACR
25 NPa	-	+	+	-	+	AC
25 Ta	-	+	+	-	+	AC

+ = Presence of a reaction.

- = Absence of a reaction.

ACR = Acid, clot and reduction.

AC = Acid and clot.

TABLE XIX.

Biochemical Reactions.

GROUP M.

Strain	Lactose	Trehalose	Mannitol	Salicin	Sorbitol	Litmus milk
1 T1	+	-	-	-	-	A
1 NP1	+	-	-	-	-	A
9 N2	+	-	-	-	-	-
9 T1	+	-	-	-	-	A
9 NP5	+	-	-	-	-	A
19 T1	+	-	-	-	-	-
22 T1	+	-	-	-	-	A
25 T1	+	-	-	-	+	A
37 T1	+	-	-	-	-	A
37 NP3	+	-	-	-	-	A
39 T1	+	-	-	-	-	A
40 T1	+	-	-	-	-	A
42 N1	+	-	-	-	-	A
42 NP1	+	-	-	-	-	A
42 T1	+	-	-	-	-	A
47 NP1	+	-	-	-	-	A
47 T1	+	-	-	-	-	A
48 NP4	+	-	-	-	-	-
48 T4	+	-	-	-	-	A
67 T1	+	-	-	-	-	A
71 T1	+	-	-	-	-	A
74 T1	+	-	-	-	-	A
75 T1	+	-	-	-	-	A
13 Ta	+	-	-	-	+	A
14 Ta	+	-	-	-	-	A
22 NPα	+	-	-	-	-	A
22 Ta	+	-	-	-	-	A

+ = Presence of a reaction

- = Absence of a reaction.

A = Acid production.

TABLE XX

GROUP C Biochemical Reaction

Strain	Lactose	Sorbitol	Trehalose	Remarks
2 NP3	+	+	-	Type 2
2 T1	+	+	-	Type 2
7 N1	+	-	+	Type 5
10 N1	+	-	+	Type 5
10 T1	+	-	+	Type 5
20 T1	+	+	-	Type 2
20 NP1	+	+	-	Type 2
23 T1	-	-	+	Type 4
23 NP1	-	-	+	Type 4
70 T5	+	+	-	Type 2
7 Nb	-	-	+	Type 4
7 Tb	-	-	+	Type 4
10 Na	+	-	+	Type 5
10 Te	+	-	+	Type 5
20 Td	+	-	+	Type 5

+ , - = Presence or absence of a reaction.

NO = Not tested.

TABLE XXI

Biochemical Reaction

GROUP L

Strain	Lactose	Trehalose	Mannitol	Salicin	Sorbitol	Litmus milk
3 NP1	+	+	-	+ slow	-	A
3 T5	+	+	-	+ slow	-	A
13 T1	+	+	-	+ slow	-	A
13 NP3	+	+	-	+ slow	-	A
18 NP1	+	+	-	-	-	A
18 T1	+	+	-	-	-	A
49 T1	+	+	-	+	-	A
9 Nc	+	+	-	+	-	A
9 Tb	+	+	-	+	-	A
9 NP6	+	+	-	+	-	A
15 Na	+	+	-	-	-	AC
15 Ta	+	+	-	-	-	AC

+, - = Presence or absence of a reaction.

A = Acid production.

AC = Acid and clot.

TABLE XXII

<u>GROUP A</u>	Biochemical Reaction		
	12 T _a	57 N ₅	57 T ₃
Sodium hippurate	-	-	-
Glucose	+	+	+
Lactose	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Sorbitol	-	-	-
Salicin	+	+	+
Raffinose	-	-	-
Mannitol	-	+	+
Inulin	-	-	-
Litmus milk	ACR	AC	AC

+, - = Presence or absence of a reaction

AC = Acid and clot

ACR = Acid, clot and reduction.

NOX-BACTERIOTIC SENSITIVITY

Method of Identification.

All strains of TABLE XXIII were prepared

signs-haemolysis (partial haemolysis of the blood

corpuscles immediately surrounding the colonies) or

no change at all on horse blood agar plate, were

recorded as non-haemolytic streptococci. The bio-

chemical reactions of all the strains was studied in

various media, sucrose, lactose, trehalose,

sorbitol, glycerol, mannitol, sorbitol and

salicin. The reaction of litmus milk, the production

of acid in peptone water, and the ability to grow

on media like milk were also studied. The

majority of the indole fermenting strains were

further tested for bile solubility and their patho-

genicity was tested by the rabbit test.

+, - = Presence or absence of a reaction.

These two tests completely eliminated any doubt that

AC = Acid and clot.

each strain were not pneumococci, as all strains

tested were non-bile-soluble and non-pathogenic for

mice, even though indole was fermented, which is an

important biochemical characteristic of pneumococci.

Result.

77.5 per cent. of the normal dogs and 76 per

cent. of the sick dogs were found to be harbouring

these organisms in one situation or another. The

carrier rates in the nose, nasopharynx and tonsils

were 28 per cent., 45 per cent. and 55.5 per cent.

respectively.

NON-HAEMOLYTIC STREPTOCOCCI

Method of identification.

All strains of streptococci which produced alpha-haemolysis (partial haemolysis of the blood corpuscles immediately surrounding the colonies) or no change at all on horse blood agar plate, were recorded as non-haemolytic streptococci. The biochemical reactions of all the strains were studied in arabinose, maltose, sucrose, lactose, trehalose, raffinose, inulin, glycerol, mannitol, sorbitol and salicin. The action on litmus milk, the production of ammonia in peptone water, and the ability to grow on media containing bile salt, were studied. The majority of the inulin-fermenting strains were further tested for bile solubility and their pathogenicity was tested by inoculation into mice. These two tests completely eliminated any doubt that such strains were not pneumococci, as all strains tested were non-bile-soluble and non-pathogenic for mice, even though inulin was fermented, which is an important biochemical characteristic of pneumococci.

Result.

77.3 per cent. of the normal dogs and 96 per cent. of the sick dogs were found to be harbouring these organisms in one situation or another. The carrier rates in the nose, nasopharynx and tonsils were 28 per cent., 46 per cent. and 65.3 per cent. respectively/

TABLE XXIV.

Percentage carrier rates for non-haemolytic Streptococci

Class	Number of dogs swabbed	No. Positive	Percent Positive
Normal	75	58	77.3
Sick	25	24	96

TABLE XXV

The frequency of non-haemolytic Streptococci in the nose, nasopharynx and tonsils

Situation	Normal Dogs			Sick Dogs			Combined		
	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%
Nose	75	21	28	25	12	48	100	33	33
Nasopharynx	50	23	46	17	9	52.9	67	32	47.8
Tonsils	75	49	65.3	25	19	76	100	68	68

respectively in normal dogs whereas they increased to 48 per cent., 52.9 per cent., and 76 per cent. in the corresponding situations in sick dogs.

An attempt was made to classify one hundred and thirty-three strains of this organism isolated from both normal and sick dogs into different groups by their physiological and, more particularly, their biochemical reactions, as recommended by Sherman (1937), since no serological method is practicable for that purpose.

As shown in Table XXVI, all the strains forming either acid and clot or acid alone in litmus milk, fermenting maltose, sucrose, and lactose, and not fermenting glycerol, mannitol, sorbitol and arabinose, were classified as *Streptococcus salivarius*. Eighty-six (64.7 per cent.) belonged to this group.

Those strains which showed no haemolysis on blood agar, clotted milk, fermented mannitol, sorbitol and salicin, and were bile-resistant were classified as *Streptococcus faecalis*. Twenty-one (15.2 per cent.) belonged to this group.

Seventeen strains (12.8 per cent.) which did not curdle milk and did not ferment lactose, but in other respects showed the same reactions as *S. salivarius* were identified as *Streptococcus equinus*.

The strains which were bile-resistant invariably curdled milk, fermented arabinose, lactose, raffinose and salicin, were recorded as *Streptococcus bovis*. Only nine strains (6.8 per cent.) belonged to this group

TABLE XXVI

NON HAEMOLYTIC STREPTOCOCCI

Biochemical Reactions

Strain	Haemolysis	Ammonia in peptone water	Growth on MacConkey Agar	Litmus milk	Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Probable species
1 NP3	Alpha	-	-	A	-	A	A	-	-	-	-	-	-	-	-	Equinus
1 T2	Alpha	-	-	A	-	A	A	-	-	-	-	-	-	-	-	Equinus
3 NP2	Alpha	-	+	AC	A	A	A	A	-	-	-	-	-	-	-	Bovis
3 T1	Alpha	-	+	A	A	A	A	A	-	-	-	-	-	-	-	Bovis
4 N5	Alpha	-	-	A	-	A	A	A	A	-	-	-	-	-	-	Salivarius
4 T2	Alpha	-	-	A	-	A	A	A	A	-	-	-	-	-	-	Salivarius
5 NP2	Alpha	-	-	A	-	A	A	A	A	-	-	-	-	-	-	Salivarius
5 T2	Alpha	-	-	A	-	A	A	A	A	-	-	-	-	-	-	Salivarius
6 NP3	Alpha	-	-	A	-	A	-	-	-	-	-	-	-	-	-	Equinus
6 T2	Alpha	-	-	A	-	A	-	-	-	-	-	-	-	-	-	Equinus
7 N4	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivarius
7 NP3	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivarius
7 T4	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivarius
8 NP7	Alpha	-	+	A	A	A	A	A	-	A	-	-	-	-	A	Bovis
8 T3	Alpha	-	-	A	A	A	A	A	-	A	-	-	-	-	A	Bovis
9 N3	Alpha	-	-	AC	-	A	A	A	-	A	-	-	-	-	A	Salivarius
8 T2	Alpha	-	-	AC	-	A	A	A	A	A	-	-	-	-	A	do.
9 NP1	Alpha	-	-	A	-	A	A	A	A	A	-	-	-	-	A	do.
10 NP2	Nil	-	-	AC	-	A	A	A	A	A	-	-	-	-	A	do.
10 T2	Nil	-	-	A	-	A	A	A	A	A	-	-	-	-	-	do.
11 NP1	Alpha	-	-	AC	-	A	A	A	-	A	A	-	-	-	-	do.
12 NP3	Nil	-	-	AC	-	A	A	A	-	A	-	-	-	-	A	do.
12 T4	Nil	-	-	AC	-	A	A	A	-	A	-	-	-	-	A	do.
13 N1	Alpha	-	-	AC	-	A	A	A	-	-	A	-	-	-	A	do.
13 T3	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	A	do.
14 NP3	Alpha	-	-	AC	-	A	A	A	-	-	-	-	-	-	-	do.
15 NP1	Alpha	-	-	AC	-	A	A	A	A	A	-	-	-	-	-	do.
15 NP2	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	A	do.
16 T4	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	A	do.
17 N1	Alpha	-	-	A	A	A	A	A	-	-	A	-	-	-	-	do.
18 T2	Nil	+	+	AC	-	A	A	A	A	-	-	A	A	A	A	Faecalis
19 NP2	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	A	Salivarius
19 T2	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	A	do.
20 N2																
20 T4																
21 N2																
21 NP1																
21 T2	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivarius
22 N2																
22 NP3																
22 T2																
23 NP3																
24 T3	Alpha	+	+	AC	A	A	A	A	A	-	-	-	A	A	A	Faecalis

TABLE XXVI (Contd.)

Strain	Strain	Haemolysis	Ammonia in peptone water	Growth on MacConkey agar	Litmus milk	Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Probable species
25	T1	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	A	Salivarius
26	NP3	Nil	+	+	AC	-	A	A	A	A	-	-	-	A	A	A	Faecalis
26	T3	Nil	+	+	AC	-	A	A	A	A	-	-	-	A	A	A	Faecalis
27	T3	Alpha	-	-	AC	-	A	A	A	-	-	A	-	-	-	-	Salivarius
28	T7	Nil	-	+	A	A	A	A	A	-	A	-	-	-	-	A	Bovis
29	T2	Alpha	-	-	A	-	A	A	A	-	A	-	-	-	-	-	Salivarius
31	NP3	Alpha	-	-	A	-	A	A	A	-	A	A	-	-	-	-	Salivarius
31	T2	Alpha	-	-	A	-	A	A	A	-	A	A	-	-	-	-	Salivarius
32	NP2	Alpha	-	-	A	A	A	A	A	-	A	-	-	-	-	A	Bovis
34	N4	Nil	+	+	AC	A	A	A	A	A	-	-	-	A	A	A	Faecalis
36	T6	Alpha	-	-	AC	-	A	A	A	-	-	A	A	-	-	-	Salivarius
37	N4	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
37	T9	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
38	N3	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
38	T6	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
39	T5	Nil	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivarius
43	N2	Nil	+	+	AC	A	A	A	A	A	-	-	A	A	A	A	Faecalis
43	T3	Nil	+	+	AC	A	A	A	A	A	-	-	A	A	A	A	Faecalis
44	T3	Nil	+	+	AC	A	A	A	A	A	-	-	A	A	A	A	Faecalis
45	N4	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Salivarius
45	T4	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
47	N1	Alpha	-	-	A	-	A	A	A	-	A	A	-	-	-	-	Salivarius
47	NP5	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
47	T4	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
49	NP1	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
50	N4	Alpha	-	-	AC	-	A	A	A	-	-	A	-	-	-	-	do.
51	N2	Alpha	-	-	A	-	A	A	A	A	A	-	-	-	-	-	do.
51	T3	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
52	N4	Nil	-	+	AC	A	A	A	A	-	A	-	-	-	-	-	Bovis
52	T6	Nil	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
53	T1	Alpha	-	-	A	-	A	A	-	-	-	-	-	-	-	-	Equinus
54	T6	Alpha	-	-	AC	-	A	A	A	A	-	A	-	-	-	-	Salivarius
55	T3	Alpha	-	-	A	-	A	A	A	-	A	-	-	-	-	-	do.
56	T6	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
57	T4	Alpha	-	-	AC	-	A	A	A	-	-	-	-	-	-	-	dp.
58	N2	Alpha	-	-	A	-	A	A	A	+	+	-	-	-	-	-	do.
58	T5	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
60	T3	Alpha	-	-	A	-	A	A	A	-	-	A	-	-	-	-	do.
61	N2	Nil	+	+	AC	A	A	A	A	A	-	-	-	A	A	A	Faecalis
61	T2	Nil	+	+	AC	A	A	A	A	A	-	-	-	A	A	A	Faecalis
62	N1	Alpha	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Equinus
62	T5	Alpha	-	-	AC	-	A	A	A	-	A	-	-	-	-	-	Salivarius
66	NP4	Alpha	-	-	A	-	A	A	A	-	A	-	-	-	-	-	do.
68	T3	Alpha	-	-	A	-	A	A	A	A	-	A	-	-	-	-	do.
70	T4	Alpha	-	-	A	-	A	A	A	-	A	-	-	-	-	-	do.

NEISSERIA (GRAM-NEGATIVE COCCI)

Method of identification.

All the gram-negative cocci isolated in this investigation were studied for their morphology, appearance of growth in broth, 5 per cent. horse blood agar, plain agar, growth at 22°C. and acid production in glucose, maltose, sucrose and lactose. On the basis of these tests it was found that most of the strains belonged to the *Neisseria catarrhalis* group and only a few to the *Neisseria pharyngis* group.

Two distinct types among *N. catarrhalis* were met with, and for convenience they were classified as *N. catarrhalis* I, and *N. catarrhalis* II. Though the biochemical reactions of both these types were the same in that both had no action on any of the sugars, they had distinct differences in their morphology and cultural characteristics. *Neisseria catarrhalis* I compared well with the *N. catarrhalis* group isolated from the human pharynx and described in all text books of bacteriology. They were spherical or slightly oval cocci arranged singly, in pairs, in tetrads or in clumps, but never in chains, short or long (Fig. 1). They produced both smooth and rough colonies on blood agar. The smooth type was round, low, convex, greyish-white, amorphous with smooth glistening surface and entire edge. The rough type was convex, whitish-grey, with slightly uneven surface/

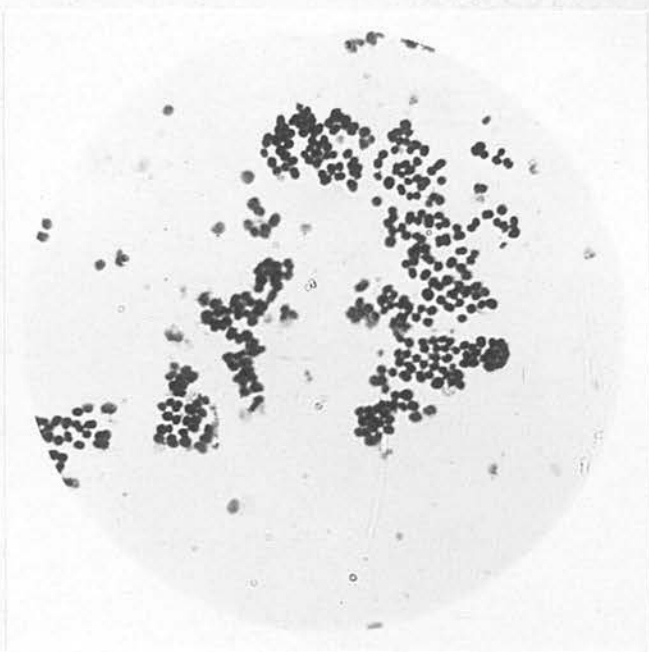


Fig. 1. - *N. catarrhalis* I,
From a blood agar plate culture,
24 hours, 37°C. (X1200).

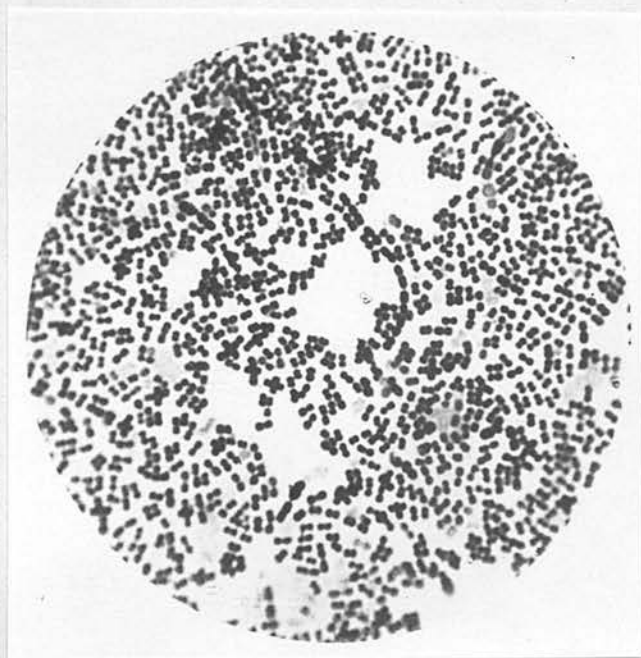


Fig. 2a - *N. catarrhalis* II.
From a blood agar culture,
24 hours, 37°C. (X1200)
Showing a tendency to form
short chains.



Fig. 2b - *N. catarrhalis* II.
From a blood agar culture,
24 hours, 37°C. (X 1200)
showing short and long chains.

surface and eaten edge, later differentiated into a raised opaque, whitish granular centre and a thinner greyish translucent periphery with a crenated edge, friable and not easily emulsifiable.

N. catarrhalis II showed distinct differences in morphology and cultural characteristics from the above group. They were spherical or slightly oval diplococci arranged in twos and short and long chains (Figs. 2a and 2b). Growth on blood agar was round, convex, reddish-white, translucent, amorphous colonies with smooth glistening surface and entire edge, butyrous consistency and easily emulsifiable. Later, the colonies became more whitish and opaque and sometimes showed a granular centre and radiate periphery. Their growth on agar was very poor, some strains even failed to grow on that medium. Similarly, growth was hardly visible in nutrient broth even on prolonged incubation. Even the addition of glucose to the broth did not improve growth. Growth on blood agar or on serum agar incubated at 22°C. was rather uncertain. Some strains grew slowly and very sparsely, while some did not grow at all. For fermentation tests of these strains, sugar medium with serum water was used. These types of cocci were isolated mostly from the nose, but occasionally from the nasopharynx and tonsils.

TABLE XXVII

Cultural and biochemical characteristics of Neisseria (Gram-negative Cocci)

Group	Morphology	Growth on Agar	Growth on Nutrient broth	Growth at 22°C.	BIOCHEMICAL			
					Glucose	Maltose	Sucrose	Lactose
N. catarrhalis I (64 strains)	Spherical or slightly oval coccus arranged singly, in pairs, in tetrads or in clumps.	+	+	+	-	-	-	-
N. catarrhalis II (21 strains)	Spherical or slightly oval coccus, arranged in pairs and in short chains.	+	+	+	-	-	-	-
N. pharyngis (10 strains)	Diplococcus arranged in dense clumps	+	+	+	A	A	A	-

Total number of strains of Neisseria isolated = 95

Neisseria catarrhalis I = 64 strains (67.4 per cent.)

Neisseria catarrhalis II = 21 strains (22.1 per cent.)

Neisseria pharyngis = 10 strains (10.5 per cent.)

Neisseria pharyngis formed a small percentage of the Gram-negative cocci isolated. They were small cocci arranged singly in pairs or in clumps. Like *catarrhalis* I, they grew freely in ordinary media. Colonies on blood agar were round, greyish-white, opaque, slightly wrinkled surface and an undulate edge. In broth, slight turbidity, granular deposit and sometimes surface pellicles were noticed. Growth was appreciable at 22°C. also. These strains fermented glucose, maltose and sucrose, but not lactose.

Results.

The incidence of these organisms in the normal and sick dogs remained practically the same. Of seventy-five normal dogs, forty-five (60 per cent.) were found to be the carriers of these organisms, whereas they were recovered from fourteen (56 per cent.) of the sick dogs. 38.7 per cent. of the normal dogs harboured them in the nose, 48 per cent. in the nasopharynx and 26.7 per cent. in the tonsils, while 32 per cent. of the sick animals were positive in the nose, 35.3 per cent. in the nasopharynx and 32 per cent. in the tonsils.

Out of the total of ninety-five strains isolated sixty-four (67.4 per cent.) were typical of *Neisseria catarrhalis* which were designated as *N. catarrhalis* I. Twenty-one (22.1 per cent.) belonged to the tentative division made in this study as *N. catarrhalis* II, and only ten strains (10.5 per cent.) belonged to the *N. pharyngis* group.

TABLE XXVIII

Percentage carrier rates for Neisseria (Gram-negative cocci)

Group	Number of dogs swabbed	Number Positive	Percent Positive
Normal	75	45	60
Sick	25	14	56
Combined	100	59	59

TABLE XXIX

The frequency of Neisseria in the nose, nasopharynx and tonsils of normal and sick dogs.

Situation	Normal Dogs		Sick Dogs		Combined	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %	Number swabbed	Positive No. %
Nose	75	29 38.7	25	8 32	100	37 37
Naso-pharynx	50	24 48	17	6 35.3	67	30 44.8
Tonsils	75	20 26.7	25	8 32	100	28 28

BACILLUS (HAEMOPHILUS) BRONCHISEPTICUS

Method of identification.

It was not possible to identify the colonies of this organism with any degree of certainty, for, though fairly characteristic, colonies of other organisms somewhat resembled them; and so in order not to miss this organism several colonies of the same type were picked from the original plate and subcultivated on to fresh plates. Any small Gram-negative motile organism growing on agar as small, round, convex, amorphous colonies with smooth glistening surface of butyrous consistency was provisionally considered to be *Haemophilus bronchisepticus*, and was further examined by biochemical and pathogenicity tests.

The biochemical reactions regarded as characteristic of *Haemophilus bronchisepticus* were, the inability to ferment any of the carbohydrates, the production of marked alkalinity in litmus milk, a dark tan colour on potato slope, growth in Koser's citrate broth, indefinite action on nitrates, and also profuse catalase production as described by Ferry, McGowan, Torrey and Rahe (loc. cit.).

The majority of the strains showing such morphological, cultural characteristics and biochemical reactions were tested for pathogenicity by the intraperitoneal inoculation of guinea pigs with 0.5 ml. - 1 ml. of a 24 hours' broth culture. Death ensued in 24 - 48 hours.

Results.

Of the seventy-five normal dogs, fourteen (18.7 per cent.) were found to be carriers of this organism, whereas it was recovered from thirteen (52 per cent.) of the sick dogs. In the normal animals it was found in 14.7 per cent. of the nasal, 10 per cent. of the nasopharyngeal and 4 per cent. of the tonsillar swabs; whereas in the sick animals, 52 per cent. of the nasal, 41.2 per cent. of the nasopharyngeal and 20 per cent. of the tonsillar swabs were positive.

All the normal dogs which harboured this organism were young animals. Five were two years old, while the remaining nine were either one year or younger. All the sick dogs studied were of immature age, except dog number 18, which was eight years old, so that again the organism was only recovered from young dogs. They were all showing early clinical symptoms of distemper or the nervous complications of the disease. On the other hand, some of the dogs showing such symptoms failed to reveal this organism.

The cultural characteristics of all the forty-four strains of this organism isolated in this investigation were uniform, except for one test, the reduction of nitrate to nitrite. Only fourteen strains were positive for this reaction, while the remaining/

TABLE XXX

Percentage carrier rates for *H. bronchisepticus* in normal and sick dogs

Group	No. of dogs swabbed	No. Positive	Percent Positive
Normal	75	14	18.7
Sick	25	13	52

TABLE XXXI

The frequency of *H. bronchisepticus* in the nose, nasopharynx and tonsils of normal and sick dogs.

Situation	Normal Dogs			Sick Dogs		
	Number swabbed	Number Positive	Percent Positive	Number Swabbed	Number Positive	Percent positive
Nose	75	11	14.7	25	13	52
Nasopharynx	50	5	10	17	7	41.2
Tonsils	75	3	4	25	5	20

remaining thirty strains had no reducing action at all. This result is quite in agreement with the observations recorded by Torrey and Rahe (loc. cit.)

Particular care was taken to differentiate this bacillus from some strains of *Bacillus faecalis alkaligenes* isolated in this investigation, which were also motile, produced no action in any of the carbohydrate media and were able to produce marked alkalinity in litmus milk. These strains, however, grew more profusely and, morphologically, were slightly thicker and longer rods than *Bacillus bronchisepticus*. Furthermore, these strains were not pathogenic for guinea pigs.

(under litmus milk) = marked alkalinity
 (under litmus milk) = growth in litmus medium
 (under litmus milk) = production of nitrate from nitrite

TABLE XXXI

Biochemical reactions of *H. bronchisepticus* isolated from normal and sick dogs

Strain	Motility	Glucose	Lactose	Mannitol	Inositol	Maltose	Dulcitol	Xylose	Arabinose	Rhamnose	Sucrose	Salicyly	Raffinose	Sorbitol	Litmus milk	Indole	M.R.	V.P.	Citrate	Nitrate	Gelatin	Potato	Ammonia	H ₂ S
5 N4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	Dark tan	-	-
11 N4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
11 NP7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
11 T7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
17 N5	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
17 T4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
23 N2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
29 NP3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
32 NP1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
33 N1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
34 N1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
34 NP2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
39 N3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
42 T2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
53 N2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
61 N2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
61 NP1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
67 N1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
72 N2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
1 Nb	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
1 Tc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
1 NPf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
5 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
6 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk _{km}	-	-	-	+	-	-	"	-	-
6 NPf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
8 Nf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
9 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
9 Tc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
9 NPe	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
12 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
12 Te	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
13 Nc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
13 NPd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
14 Nc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
14 Te	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
15 Nc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
15 NPe	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
17 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
17 NPf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
19 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
19 NPf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-
23 Ne	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
23 Tf	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	+	-	"	-	-
15 Nb	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	"	-	-

Alk (under litmus milk) = Marked alkalinity .
 + (under citrate) = growth in citrate medium. - (under gelatin) = no liquefaction.
 + (under nitrate) = reduction of nitrate to nitrite.

COLIFORM BACILLI

Result. Percentage carrier rates for coliform bacilli in normal and sick dogs.

Organisms belonging to this group were isolated from sixteen (21.3 per cent.) of the seventy-five normal dogs and from twelve (48 per cent.) of the twenty-five sick dogs. Their frequencies in the nose, nasopharynx and tonsils of normal dogs were 14.7 per cent., 4 per cent. and 5.3 per cent. respectively, whereas they increased in the sick dogs to 20 per cent., 17.6 per cent. and 28 per cent. in the corresponding parts.

Out of the thirty-two strains of this organism isolated from normal and sick dogs, fourteen were almost typical of the *B. coli* type in not fermenting inositol, producing indole, giving positive methyl red reaction, and failing to utilise citrate.

Twelve strains were identified as *B. lactis aerogenes*, an atypical strain of *B. coli*, by their ability to ferment inositol, absence of indole production, positive V.P. and negative M.R. reaction, and by their ability to grow in citrate medium.

One exception in this group (strain 4LN₄) was noted, giving positive M.R. and negative V.P. reaction, corresponding to the pneumobacillus type. Most of the strains in this group showed small capsules. Six strains were grouped as *B. cloacae* type on account of their ability to liquefy gelatin.

TABLE XXXIII

Percentage carrier rates for Coliform bacilli
in normal and sick dogs.

Group	No. of dogs swabbed	Number Positive	Percent Positive
Normal	75	16	21.3
Sick	25	12	48
Combined	100	28	28

TABLE XXXIV

The frequency of Coliform bacilli in the nose, nasopharynx
and tonsils.

Situation	Normal Dogs		Sick Dogs		Combined	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %	Number swabbed	Positive No. %
Nose	75	11 14.7	25	5 20	100	16 16
Nasopharynx	50	2 4	17	3 17.6	67	5 7.4
Tonsils	75	4 5.3	25	7 28	100	11 11

TABLE XXXV.

Showing biochemical reactions of various strains of Coliform organisms isolated from normal and sick dogs and their probable identity

Strain	Motility	Glucose	Lactose	Mannitol	Inositol	Maltose	Dulcitol	Xylose	Arabinose	Rhamnose	Sucrose	Salicin	Raffinose	Sorbitol	Indole	M.R.	V.P.	Citrate	T.M.	Gelatin	Probable Identity
3 N5	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	B. cloacae type
6 N3	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	B. lactis aerogenes.
9 N5	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	" "
8 T5	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	" "
10 N4	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. coli - typical
15 N3	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	B. lactis aerogenes
20 N4	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. coli - typical
23 N2	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
28 T9	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. lactis aerogenes
34 NP4	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	+	B. cloacae type
41 N4	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	B. lactis aerogenes
46 T6	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	" "
52 N6	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	B. cloacae type
55 N3	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	" "
63 N.	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. coli - typical
64 NP	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	+	+	AC	-	B. lactis aerogenes
69 TA	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	" "
1 Te	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	B. cloacae type
4 Ng	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	B. lactis aerogenes
8 Nf	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. coli-typical
9 NPo	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
9 Ta	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
11 Te	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
12 Tf	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
14 Tf	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
16 Nd	-	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	-	B. lactis aerogenes
17 NPe	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	" "
18 Nf	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	AC	+	B. cloacae type
18 Tg	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	B. coli typical
19 NPa	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
19 Ta	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "
24 NPf	+	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	+	+	-	-	AC	-	" "

DIPHTHEROID BACILLI

Only two species of diphtheroid bacilli were isolated in this study:-

1. Gram-positive short rods, showing no meta-chromatic granules, growing fairly well in ordinary media, having no action on any of the carbohydrates, were identified as *Corynebacterium hofmanni* (*Bacillus* of Hofmann).

2. Organisms resembling *Corynebacterium xerosis* formed the second group. They were non-motile, slightly pleomorphic, Gram-positive rods of medium size, slightly curved, clubbed and segmented, frequently arranged in small clusters showing clear metachromatic granules. Growth in broth was moderate with slight turbidity and granular deposit on 24 hours' incubation. On agar, minute raised, round, smooth, almost transparent, colonies were seen after 48 hours' incubation. Growth was more profuse on media containing blood or serum. No haemolysis was seen on blood agar plate. All the strains produced acid without gas in glucose, maltose and sucrose; other sugars were not attacked by any of the strains. Litmus milk was not changed. Indole was not produced. They were non-pathogenic to laboratory animals.

Of seventy-five normal dogs, only fourteen (18.7 per cent.) showed diphtheroid bacilli (8 per cent. *C. hofmanni* and 10.7 per cent. *C. xerosis*). Similarly,

TABLE XXXVI

Percentage carrier rates for diphtheroid bacilli
in normal and sick dogs.

Group	Number of dogs swabbed	Number Positive			Percent Positive		
		C. hofmanni	C. xerosis	Total	C. hofmanni	C. xerosis	Total
Normal	75	6	8	14	8	10.7	18.7
Sick	25	4	3	7	16	12	28
Combined	100	10	11	21	10	11	21

Similarly, of twenty-five sick dogs, seven (28 per cent.) were found positive for this group of organisms (16 per cent. *C. hofmanni* and 12 per cent. *C. xerosis*).

The carrier rates in the nose, nasopharynx and tonsils were 14.7 per cent., 6 per cent. and 2.7 per cent. respectively in normal dogs, whereas it increased to 16 per cent., 11.8 per cent. and 4 per cent. in the corresponding parts in sick dogs. The frequency of *C. xerosis* was greater than that of *C. hofmanni* in the nose, both in normal and sick dogs; while only 4 per cent. of the normal dogs were positive for *C. hofmanni* in the nose, 10.7 per cent. showed *C. xerosis* in the same situation. Similarly, while *C. hofmanni* was found in the nose of one (4 per cent.), *C. xerosis* was isolated from the nose of three (12 per cent.) sick dogs. *C. xerosis* was not found in the tonsils of any of the normal or sick dogs.

TABLE XXXVII

The frequency of diphtheroid bacilli in the nose, nasopharynx and tonsils of normal and sick dogs

Situation	Normal Dogs				Sick Dogs				Combined											
	Number swabbed	Number Positive		Percent Positive	Number swabbed	Number Positive		Percent Positive	Number swabbed	Number Positive		Percent Positive								
		C. hoffmanni	C. kerosits			Total	C. hoffmanni			C. kerosits	Total		C. hoffmanni	C. kerosits	Total					
Nose	75	3	8	4	10.7	14.7	25	1	3	4	4	12	16	100	4	11	15	4.5	3	7.5
Nasopharynx	50	2	1	4	2	6	17	1	1	2	5.9	5.9	11.8	67	3	2	5	4.5	3	7.5
Tonsils	75	2	-	2.7	-	2.7	25	3	-	3	4	-	4	100	5	-	5	5	-	5

BACILLUS SUBTILIS GROUP

All aerobic Gram-positive, spore-bearing bacilli were included in this group. They were all motile, non-capsulated, formed spores abundantly, produced uniform turbidity with granular growth and often surface scum in broth. On agar the colonies gave a granular mealy appearance or membranous and thrown into wrinkles.

Their classification into *B. subtilis*, *B. mesentericus*, *B. vulgatus* and *B. megatherium* on the basis of their biochemical reactions, as shown in Table XXXIX, was purely arbitrary, as biochemically there were no striking variations between these groups. All of them liquefied gelatin, reduced nitrates, and produced abundant catalase. The majority of them reduced, coagulated and peptonised litmús milk in varying degrees; two strains (26 N₄ and 30 N₂) only reduced and peptonised, and failed to coagulate. Fermentation of glucose, maltose, sucrose, and trehalose was constant with all strains.

These organisms were isolated from twelve (16 per cent.) of the normal dogs and from five (20 per cent.) of the sick dogs, all from the nasal swabs, excepting in one sick dog (No. 20), in which, in addition to the nose, it was also found in the tonsils swab.

TABLE XXXVIII

The frequency of ~~isolation~~ of Bacillus Subtilis group in normal and sick dogs.

Group	No. of dogs swabbed	Number Positive	Percent Positive
Normal	75	12	16
Sick	25	5	20
Combined	100	17	17

TABLE XXXIX

Biochemical reactions of Bacillus Subtilis group

Strain	Glucose	Lactose	Mannitol	Maltose	Dulcitol	Arabinose	Rhamnose	Sucrose	Salicin	Trehalose	Dextrin	Xylose	Litmus milk	Indole	M.R.	V.P.	NH ₃	H ₂ S	Gelatin	Nitrate	Catalase	Probable identity
1 N3	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
22 N4	A	-	A	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. mesentericus
25 N3	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. Megatherium
26 N4	A	-	-	A	-	A	-	A	A	A	A	A	RP	-	-	-	+	-	+	+	+	B. vulgatus
30 N2	A	-	-	A	-	A	-	A	A	A	A	A	RP	-	-	-	+	-	+	+	+	B. vulgatus
35 N2	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. vulgatus
36 N2	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
38 N1	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
39 N1	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
49 N3	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
57 N6	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
64 N5	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. megatherium
5 Nc	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
8 Te	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
12 Ne	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis
19 Ne	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. mesentericus
20 Nd	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. mesentericus
20 Tf	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. megatherium
20 Tf	A	-	-	A	-	A	-	A	A	A	A	A	RCP	-	-	-	+	-	+	+	+	B. subtilis.

+ under gelatin = Liquefaction.
 + " nitrate = Reduction.
 RCP = Reduced, coagulated and peptonised
 RP = Reduced and peptonised.

BACTERIUM ALKALIGENES

All Gram-negative rods of variable size, motile or non-motile, growing profusely or moderately on ordinary media, including MacConkey's bile salt agar, having no fermentative property of any of the commonly used carbohydrates, either showing no change or producing marked alkalinity in litmus milk, not forming indole, and non-pathogenic to laboratory animals, were included in this group.

Fifty-eight strains belonging to this group were isolated from normal and sick dogs (34 strains from normal and 24 from sick dogs). Only eighteen strains were typical of *Bacterium faecalis alkaligenes* described by Petruschky (1896). They were actively motile, Gram-negative, slender rods, slightly longer and thinner than those of *Bacterium coli*. The colonies on agar were also slightly flatter than those of *B. coli* and more contoured with a raised centre and undulate edge. Litmus milk was rendered strongly alkaline, and a characteristic brown colour was produced on potato. Growth was very profuse both on solid and liquid media. In broth, surface ring and pellicle were formed. Growth on MacConkey's medium was profuse with the formation of a yellow zone around each colony. Sixteen of these strains grew on citrate medium, whereas two (22 T_c and 25 N_c) failed to grow on that medium.

TABLE XL

Percentage carrier rates for *Bacterium alkaligenes*.

Group	No. of dogs swabbed	Number Positive	Percent Positive
Normal	75	27	36
Sick	25	15	60
Combined	100	42	42

TABLE XLI

The frequency of *Bacterium alkaligenes* in the nose, nasopharynx and tonsils

Situation	Normal Dogs		Sick Dogs			Combined.	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %	Number swabbed	Positive No. %	
Nose	75	16 21.3	25	10 40	100	26 26	
Nasopharynx	50	3 6	17	6 35.3	67	9 13.4	
Tonsils	75	15 20	25	8 32	100	23 23	

The remaining forty strains were distinctly different from the above in their morphology and cultural characteristics. They were rather similar to the types described by Nyberg (1935) and named *Bacterium alkaligenes*. They were short, thick bacilli, non-motile, not producing appreciable change in litmus milk and no clear pigmentation on potato. The colonies on agar plate were somewhat similar, and the growth was not so profuse as in the motile type described by Petruschky. Growth in broth was moderate without the formation of surface ring and pellicle. Among these forty strains, fifteen failed to grow in citrate medium, eight showed slight alkalinity in litmus milk, and four showed slight brown pigmentation on potato.

Organisms belonging to this group were isolated from twenty-seven (30 per cent.) of the normal dogs, and from fifteen (60 per cent.) of the sick dogs. Their frequency in the nose, nasopharynx and tonsils of normal dogs was 21.3 per cent., 6 per cent. and 20 per cent. respectively, while it increased to 40 per cent., 35.3 per cent. and 32 per cent. in the corresponding parts in sick dogs.

TABLE XLII (Contd.)

Strain	Motility	Glucose	Lactose	Mannitol	Inositol	Maltose	Dulcitol	Xylose	Arabinose	Sucrose	Salicin	Raffinose	Sorbitol	Hamnose	Litmus milk	Indole	M.R.	V.P.	Citrate	Nitrate	Gelatin	NH ₃	H ₂ S	Potato
61 N3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
63 T3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67 N3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69 N4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 Nc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk	-	-	-	+	-	-	-	-	Brown
1 NFe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Sl. Alk.	-	-	-	-	-	-	-	-	Brown
3 Na	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
3 Tg	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
7 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
7 NPd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9 NPF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
9 Tg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
10 N1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
11 Tf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12 Tg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14 Tc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Sl. Alk.	-	-	-	+	-	-	-	-	Sl. brown
17 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Sl. Alk.	-	-	-	+	-	-	-	-	Sl. brown
18 f	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	-	-	-	-	-	Brown
20 Td	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22 Nc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22 Tc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	-	-	-	-	-	Brown
23 Nc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23 NPd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
24 Nd	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	+	-	-	-	-	Brown
24 NPe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Sl. Alk.	-	-	-	+	-	-	-	-	Brown
24 Tb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 Nc	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Alk.	-	-	-	-	-	-	-	-	Brown
25 NPd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

+ in motility column = motile. + in citrate column = utilisation of citrate.
 ALK (under litmus milk) = distinct alkali production.
 Sl. Alk " = slight alkali production.

"
FRIEDLANDER'S BACILLUS

Five strains of this organism were isolated from four sick dogs. One (8 T_b) from the tonsils of a dog showing early symptoms of distemper, two (16 N_a and 16 T_a) from the nose and tonsils of a dog which was very anaemic and debilitated, one (20 N_c) from the nose of a dog having convulsions, and one more (23 N_f) from the nose of a dog with chorea. All the strains were rather uniform in their cultural and biochemical characteristics. They grew with characteristic large mucoid colonies on agar plate, not very unlike coliform bacilli. All of them had small capsules and were all non-motile. They showed characteristic nail-headed growth in gelatin stab - a circular convex growth on the surface with a fili-form growth in the stab, the whole resembling a round headed nail. (Fig. 3). Only two strains (16 N_a and 16 T_a) were positive for indole, the rest were negative. All were positive for methyl red reaction and negative for V.P. Two strains (20 N_c and 23 N_f) did not ferment inositol. They were all mildly pathogenic to mice. 0.5 c.c. to 1.0 c.c. of broth culture injected intraperitoneally killed the mice in 24-48 hours, and the organism was recovered in pure culture from the heart blood, liver and spleen.

The Influence of Friedlander's bacillus on the
upper respiratory tract of man.

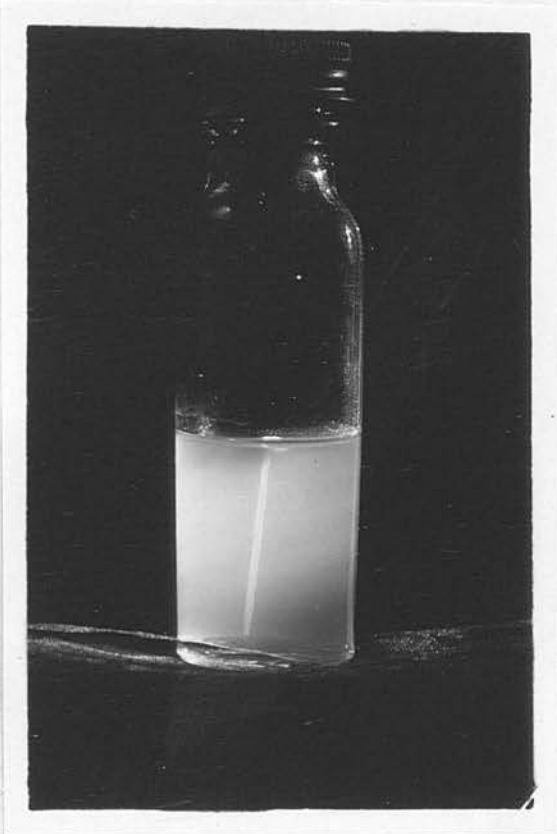


Fig. 3.

Friedlander's bacillus (8T_b)

Gelatin stab culture, 10 days, 22°C.

showing nail-headed growth.

TABLE XLIII

The Incidence of Friedländer's bacillus in the upper respiratory tracts of dogs.

Group	No. of dogs swabbed	No. Positive	Percent Positive
Normal	75	-	-
Sick	25	4	16
Combined	100	4	4

TABLE XLIV

The frequency of Friedländer's bacillus in the nose, nasopharynx and tonsils

Situation	Normal Dogs		Sick Dogs	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %
Nose	75	- -	25	2 8
Nasopharynx	50	- -	17	1 6
Tonsils	75	- -	25	2 8

TABLE XLV

Biochemical and other characteristics of Friedlander's bacillus isolated from sick dogs.

	8 T _b	16 N _a	16 T _a	20 N _c	23 N _f
Capsule	Small	Small	Small	Small	Small
Motility	Nil	Nil	Nil	Nil	Nil
Celatin stab	Typical nail-headed growth	Nail-headed growth	Nail-headed growth	Nail-headed growth	Nail-headed growth
Catalase	+	+	+	+	+
Indole	-	+	+	-	-
M.R.	+	+	+	+	+
V.P.	-	-	-	-	-
Nitrates	+	+	+	+	+
NH ₃	+	+	+	+	+
H ₂ S	-	-	-	-	-
M.B.	Slightly reduced	Reduced	Reduced	Slightly reduced	Reduced
L.M.	Acid	Acid & clot	Acid & clot	Acid & clot	Acid & clot
Citrate	+	+	+	+	+
Glucose	Ag	Ag	Ag	Ag	Ag
Lactose	Ag	Ag	Ag	Ag	Ag
Mannitol	Ag	Ag	Ag	Ag	Ag
Inositol	Ag	Ag	Ag	-	-
Maltose	Ag	Ag	Ag	Ag	Ag
Dulcitol	-	-	-	-	-
Sucrose	Ag	Ag	Ag	Ag	-
Salicin	Ag	Ag	Ag	Ag	Ag
Arabinose	Ag	Ag	Ag	Ag	Ag
Raffinose	Ag	Ag	Ag	Ag	Ag
Trehalose	Ag	Ag	Ag	Ag	Ag
Inulin	-	-	-	-	-
Dextrin	Ag	Ag	Ag	Ag	Ag
Rhamnose	Ag	Ag	Ag	Ag	Ag
Xylose	Ag	Ag	Ag	Ag	Ag
Sorbitol	Ag	Ag	Ag	Ag	Ag

Ag = Acid and gas

HAEMOPHILUS CANIS.

Any Gram-negative cocco-bacillus or small, slender, pleomorphic bacillus, not growing on nutrient agar or in broth, but growing slowly on blood agar and chocolate agar, showing small, round, convex, colourless or slightly opaque colonies was regarded as haemophilic, and it was further tried in various other media, such as glucose broth, serum broth, serum agar, liver infusion agar, solidified serum and Dorset's egg medium, before confirming it as such.

The accessory growth factors of these strains were studied by testing their ability to grow on the following media, as recommended by Thjotta and Avery, (1921), Rivers (1922) and Fildes (1924).

- (a) Agar with haematin, representing the auto-clave stable X factor (2 per cent.).
- (b) Agar with yeast extract, representing the auto-clave labile Y factor (10-15 per cent.).

Haematin and yeast extract were prepared by the following methods:

Haematin: Red blood cells from 100 c.c. of blood were washed in physiological salt solution, laked with distilled water and precipitated with 95 per cent. alcohol. The precipitate was boiled with weak acid alcohol (H_2SO_4) until colourless and then filtered. The filtrate was partially saturated with NaCl and filtered. The precipitate was washed on the filter with distilled water, dissolved in warm acid/

acid alcohol, re-precipitated with NaCl, filtered, washed, taken up in acid alcohol, made slightly alkaline (pH 7.4) with NaOH and brought up to a volume of 100 c.c. with 95 per cent. alcohol. This was added to any basic medium in quantities of 2 c.c. per 100 c.c. and always autoclaved.

Yeast extract: 100 grammes of brewer's yeast were emulsified in 400 c.c. of distilled water. Since vitamins will stand boiling better in acid than in an alkaline solution, the reaction of the suspension of yeast cells was adjusted to pH 4.6, boiled for ten minutes, and then allowed to settle at room temperature. The clear supernatant extract was pipetted off, filtered through paper and sterilised by means of a Mandler filter. This clear sterile extract was stored in an ice box and the necessary quantity added to the medium immediately before use.

Haemopeptone water, recommended by Rivers and Kohn (loc. cit.), was used as a liquid medium for examining its motility and testing its indole production. The same medium with 0.1 per cent. potassium nitrate added to it was used for testing nitrate reduction. Sugar medium too, recommended by the same workers, was used for the fermentation test.

All/

All the haemophilic strains isolated in this investigation grew on agar with haematin, but not on agar with yeast extract, showing that "X" only, not "Y" was required as the accessory growth factor. The strains were non-motile, positive for indole and nitrate reduction. All of them fermented glucose, galactose, mannitol and sucrose, but not lactose, maltose or dextrin.

Thus, they were all identified as *Haemophilus canis* by their morphology, cultural characteristics and biochemical reactions.

This organism was isolated only from nine (12 per cent.) of the seventy-five normal and from two (8 per cent.) of the twenty-five sick dogs. Of the ten strains isolated from normal dogs, four were from the nose, three from the nasopharynx and three from the tonsils. Similarly, of the four strains from the sick dogs, two were from the nose, one from the nasopharynx and one from the tonsils.

TABLE XLVI

Percentage carrier rates for Haemophilus canis in normal and sick dogs.

Group	Number of dogs swabbed	Number Positive	Percent Positive
Normal	75	9	12
Sick	25	2	8
Combined	100	11	11

TABLE XLVII

The frequency of Haemophilus canis in the nose, nasopharynx and tonsils

Situation	Normal Dogs			Sick Dogs			Combined		
	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%	Number swabbed	Positive No.	%
Nose	75	4	5.3	25	2	8	100	6	6
Nasopharynx	50	3	6	17	1	5.9	67	4	6
Tonsils	75	3	4	25	1	4	100	4	4

PROTEUS SPECIES

All Gram-negative, motile, pleomorphic organisms producing amoeboid colonies on nutrient agar plate, fermenting glucose with acid and gas but not lactose, liquefying solid serum and gelatin, were included in this group. For confirmation, other tests, such as indole production, nitrate reduction, decomposition of urea into ammonia, formation of hydrogen sulphide in lead acetate agar medium, production of catalase, ammonia in peptone water, and action on litmus milk, were also conducted.

All the twenty-three strains isolated in this investigation liquefied gelatin, all but two (19N₂ and 37T₈) liquefied solid serum as well. All the strains decomposed urea into ammonia, only six strains failed to produce indole. Similarly, two strains were negative for methyl red reaction (37T₄ and 48N₅); only two strains (24N_b and 24NP_c) were recorded as showing positive V.P. reaction. Nitrate reduction was rather constant. All were positive, excepting two strains (19N₂ and 35NP₅) which were recorded as having doubtful reaction. Production of ammonia in peptone water ^{was} rather constant, excepting in two strains (19N₂ and 2T_c) in which the reaction was doubtful. Only one strain (68N₂) failed to produce hydrogen sulphide. All strains were positive for Catalase. Action on litmus milk was rather variable/

variable. The majority of the strains produced alkali and peptonisation. All produced acid and gas in glucose, and all but two in sucrose as well.

Nearly 50 per cent. of the strains fermented maltose, while none of them fermented lactose, mannitol or dextrin.

These organisms were isolated from twelve (16 per cent.) of the seventy-five normal dogs and from seven (28 per cent.) of the twenty-five sick dogs. The frequency in the nose, nasopharynx and tonsils of normal dogs was 6.7 per cent., 4 per cent. and 6.7 per cent. respectively, whereas in the sick it rose to 20 per cent., 11.8 per cent. and 16 per cent. respectively in the corresponding parts.

TABLE XLIX

Percentage carrier rates for Proteus Bacilli in dogs.

Group	Number of dogs swabbed	Number Positive	Percent Positive
Normal	75	12	16
Sick	25	7	28
Combined	100	19	19

TABLE L

The frequency of Proteus Bacilli in the nose, nasopharynx and tonsils of normal and sick dogs

Situation	Normal Dogs		Sick Dogs		Combined	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %	Number swabbed	Positive No. %
Nose	75	5 6.7	25	5 20	100	10 10
Nasopharynx	50	2 4	17	2 11.8	67	4 6
Tonsils	75	5 6.7	25	4 16	100	9 9

LEPTOTRICHIA SP.

All the organisms included in this group were not uniform in their morphology and staining reactions, even though they did not show much marked difference in their cultural and biochemical characteristics.

Morphology.

Smears from the growth on solid media showed unbranched filaments which were usually unsegmented, straight or curved and sometimes intertwined in tangled masses. Ends were square or rounded or clubbed at one end. Some filaments were twisted, and such irregular and swollen forms were often encountered (Fig. 4). Individual organisms varied in length from 2 to 250 microns and in width from 0.6 to 1.4 microns. Short and bacillary forms were most prominent in smooth colonies (Fig. 5). and long filamentous forms in rhizoid colonies (Fig. 6). When grown in liquid media, they were mostly broken and only short bacillary forms were seen.

Staining reactions were rather indefinite. Generally they were Gram-positive, but sometimes decolorised quickly. Very irregular staining was noticed in some in which a few organisms retained Gram's staining while some others in the same field showed a Gram-negative reaction. Often a single element retained Gram's staining in some parts while in other parts it was completely decolorised. Some strains/

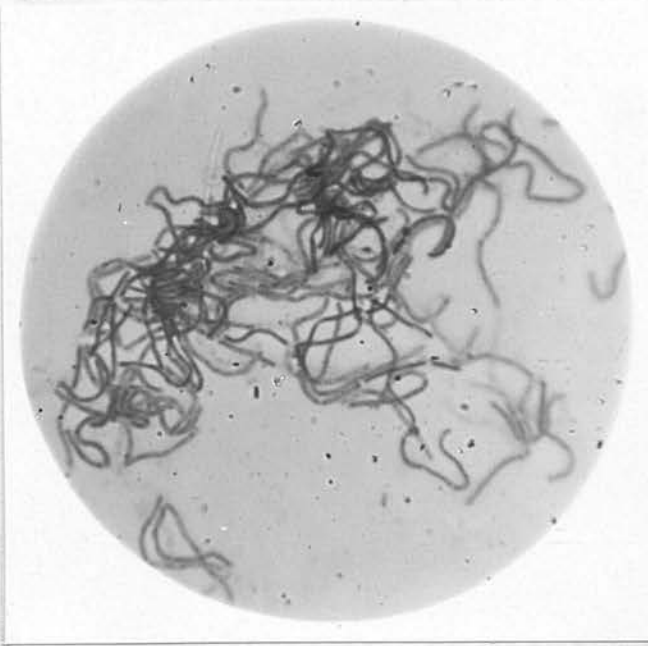


Fig.4- *Leptotrichia* sp.
From blood agar culture,
48 hours, 37°C., showing
filaments of irregular
shape. (X 1200).



Fig. 5. - *Leptotrichia* sp.
From blood agar culture,
48 hours, 37°C., showing
short and bacillary forms.
(X 1200)



Fig. 6. - *Leptotrichia* sp.
From blood agar culture, 48 hours,
37°C., showing long filamentous
forms (X 1200).

strains showed round granules in all elements by Gram's staining. By methylene blue staining old cultures showed clear vacuoles in their elements. They were all non-acid fast, non-sporing and non-motile.

Growth on blood agar. Both smooth and rough colonies were observed on blood agar plates. Smooth colonies were round, convex, entire, 1.5 - 2.4 mm. in diameter, creamy white, butyrous and easily emulsifiable (Fig. 7). Rough colonies were greyish-white, circular, having a raised granular centre and a flat peripheral portion, across which tangled filamentous processes radiated to give a rhizoid appearance (Fig.8). They were between 1 - 2 mm. in diameter. Some colonies were raised at the centre, giving an umbonate appearance with an even rounded edge and fine filamentous outgrowths (Fig. 9). No haemolysis.

Plain agar. Growth on plain agar was poor. Small pinhead-like colonies were observed after four days' incubation at 37°C.

MacConkey agar - No growth.

Broth. Growth was slow, a scanty granular sediment appearing after 3-4 days. The granules settled down at the bottom, leaving the medium clear.

Gelatin stab. No growth was noticed.

Löffler's serum. In forty-eight hours, slightly raised, whitish, glistening, confluent growth, which increased on further incubation. No liquefaction even on prolonged incubation.

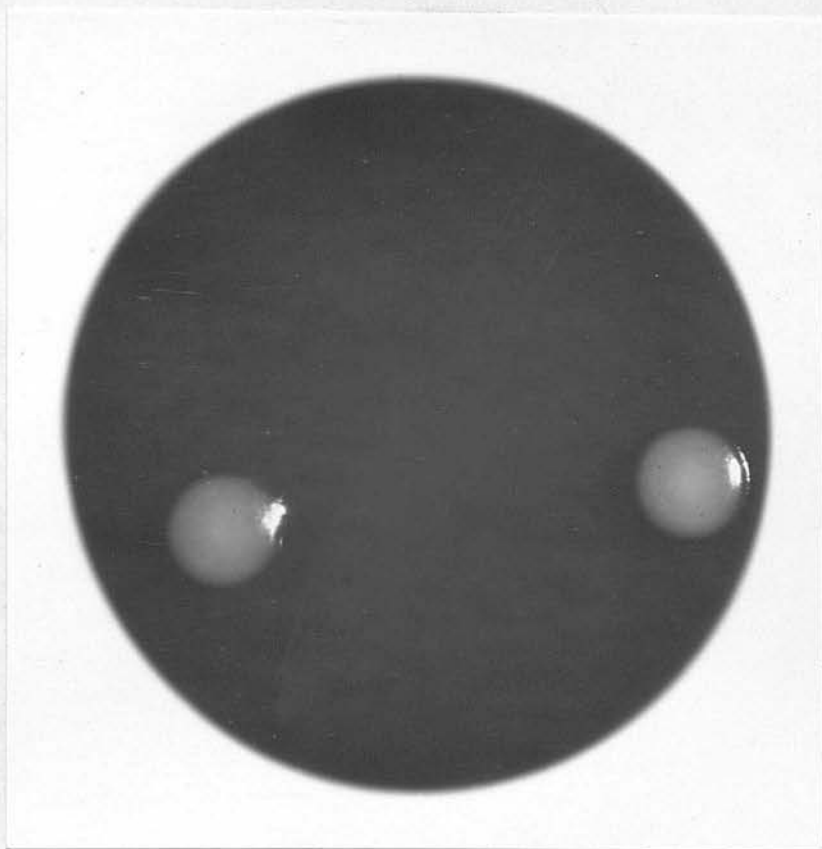


Fig. 7. - *Leptotrichia* sp.
Smooth type of colonies on blood agar,
4 days, 37°C. (X15).

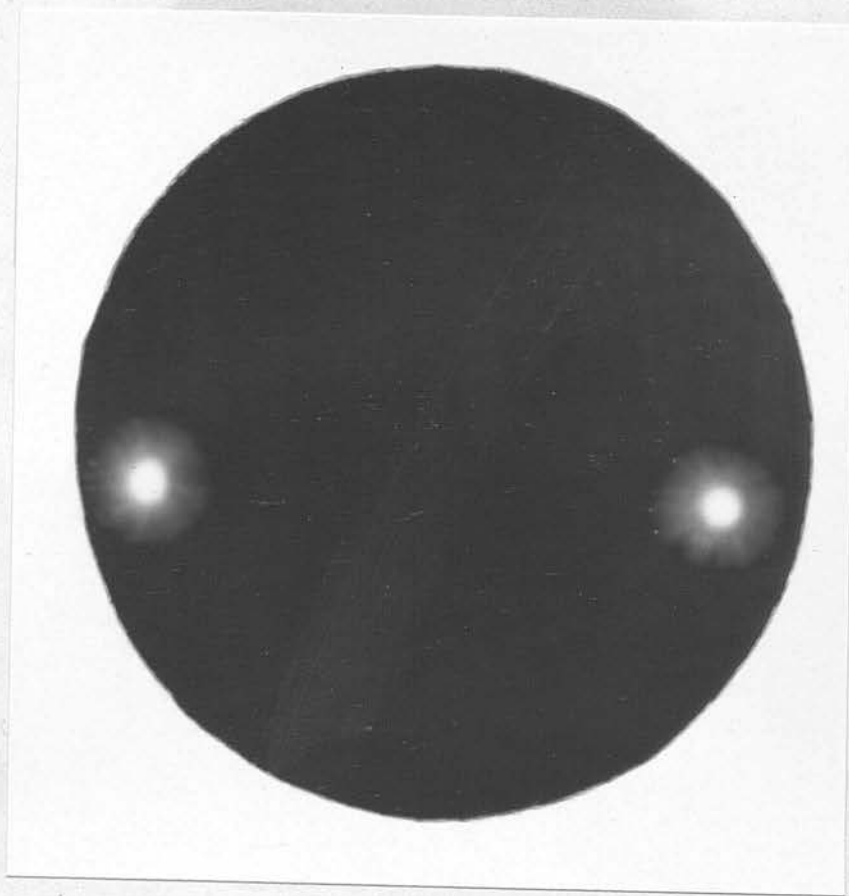


Fig. 8. - *Leptotrichia* sp.
Rough type of colonies on blood agar,
4 days, 37° C. (X10)

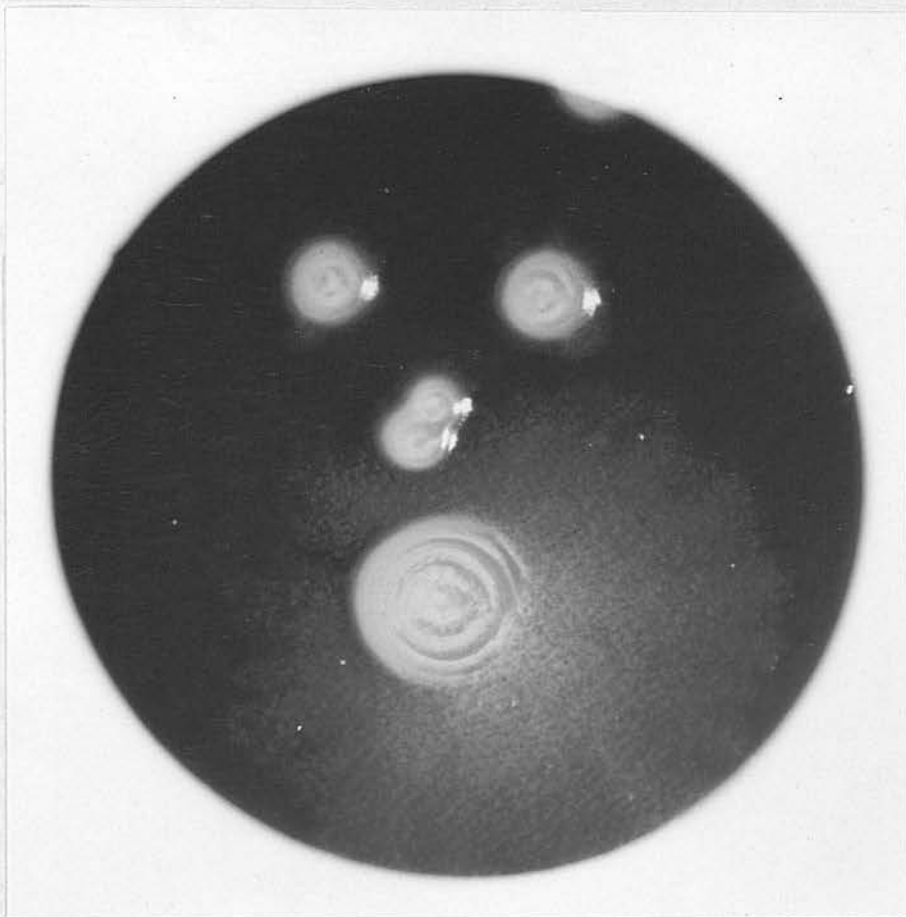


Fig. 9. - *Leptotrichia* sp.
Surface colonies on blood agar,
4 days, 37 C., showing raised
granular centre and fine filamentous
outgrowths. (X15).

Potato slope - No growth was observed.

Metabolism.

Aerobic. Seventy-five per cent. of the strains did not grow at all under strict anaerobic conditions. About twenty-five per cent. showed poor growth under anaerobic culture. Optimum temperature was 37°C. No growth was observed at room temperature. Slow and poor growth at 22°C.

Biochemical. No fermentation of sugars. No change in litmus milk. Indole -; M.R. -: V.P. -; Nitrate reduction -; NH₃ -: H₂S -; Catalase $\frac{+}{-}$; M.B. reduction -.

Pathogenicity.

Injections of 48 hours' serum broth culture of a few strains of these organisms into guinea-pigs, rabbits and mice, failed to produce any progressive pathological reaction.

Results.

Of the seventy-five normal dogs, eighteen (24 per cent.) and of the twenty-five sick dogs, seven (28 per cent.) were positive for this group of organisms. They were not once found in the nose of either normal or sick dogs, but their frequencies in the nasopharynx and tonsils of normal dogs were 18 per cent. and 24 per cent. respectively, while they were 13 per cent. and 24 per cent. in the corresponding situations in sick dogs.

TABLE LI.

Percentage carrier rates for *Leptotrichia* sp. in normal and sick dogs.

Group	No. of dogs swabbed	Number Positive	Percent Positive
Normal	75	18	24
Sick	25	7	28
Combined	100	25	25

TABLE LII

The frequency of *Leptotrichia* sp. in the nose, nasopharynx and tonsils

Situation	Normal Dogs		Sick Dogs		Combined	
	Number swabbed	Positive No. %	Number swabbed	Positive No. %	Number swabbed	Positive No. %
Nose	75	- -	25	- -	100	- -
Nasopharynx	50	9 18	17	4 23.5	67	13 19.4
Tonsils	75	18 24	25	6 24	100	24 24

UNIDENTIFIED.

During the course of this investigation several unknown Gram-negative organisms were constantly met with, and on the basis of their morphology, cultural and biochemical characteristics and pathogenicity tests, they were mainly divided into three groups as described below.

Unidentified group (a).

Morphology. The organisms of this group were small rods varying in length from 0.8 to 3 microns and in breadth from 0.4 to 0.8 micron, arranged singly in two's and small groups. Some were coccoid and the rest had a straight axis and rounded ends. A few filaments were occasionally seen. In some places they looked like diplobacilli. They had no capsule. They were non-motile at 37°C. and also at 22°C.

Growth on blood agar plate. Twenty-four hours at 37°C. - round, convex, entire, smooth, about 1-3 mm. in diameter. The colonies were somewhat greenish, but on further incubation they became definitely green and even chocolate coloured. There was a zone of clear beta haemolysis around these colonies. The growth was sticky and gum-like.

Agar plate. Opaque, glistening mucoid colonies; no pigmentation.

Chocolate agar. Profuse growth with chocolate pigmentation.

Agar stroke. Slight growth only at the surface.

Gelatin stab. Napiform liquefaction in about a week.

Broth. Twenty-four hours at 37°C. - Moderate uniform turbidity. No surface growth and no deposit, but on further incubation a slight mucoid deposit was seen.

Löffler's serum. Liquefaction with greenish-brown tinge.

Potato. Thin growth - dark brownish.

MacConkey agar plate. - No growth.

Metabolism. Aerobic. No growth under strict anaerobic conditions. Optimum temperature 37°C. Slow growth at 22°C. No growth at all at room temperature. Slight or no pigmentation when grown at 22°C.

Biochemical. No action on any of the carbohydrates
Catalase positive; Indole positive; M.R. negative;
V.P. negative; Nitrate reduction negative; NH₃ negative
H₂S negative; M.B. negative. Litmus milk - at first slightly acid then changing to slightly alkaline, decolorised and peptonised. Often clots were formed, which gradually became slightly yellowish.

Ammonia from urea. Urea was decomposed into ammonia. It was tested by inoculating a tube of buffered urea-phosphate medium with the culture (Ferguson and Hook, 1943) and after incubating for 24 hours tested for alkaline production with bromo-thymol blue indicator.

Pathogenicity. Non-pathogenic to mice, guinea pigs and rabbits.

Results. The percentage carrier rates for this group of organisms in the nose, nasopharynx and tonsils of normal and sick dogs did not vary to any appreciable degree. Of seventy-five normal dogs, forty-five (60 per cent.) and of twenty-five sick dogs, sixteen (64 per cent.) were positive in one situation or another. They were most frequently found in the tonsils and nasopharynx and occasionally in the nose. 58.2 per cent. of the dogs harboured them in the nasopharynx, 61 per cent. in the tonsils, whereas only 8 per cent. showed in the nose.

The Frequency of

Situation	Normal Dogs		Sick Dogs
	Number Examined	Positive No.	
Nose	15	5	20
Nasopharynx	50	29	25
Tonsils	75	45	25

TABLE LIII

Percentage carrier rates for unidentified group (a)
in normal and sick dogs.

Group	Number examined	Number Positive	Percent Positive
Normal	75	45	60
Sick	25	16	64
Combined	100	61	61

TABLE LIV

The frequency of unidentified group (a) in the nose,
nasopharynx and tonsils.

Situation	Normal Dogs		Sick Dogs			Combined		
	Number examined	Positive No. %	Number examined	Positive No. %	Number examined	Positive No. %		
Nose	75	6 8	25	2 8	100	8 8		
Nasopharynx	50	29 58	17	10 59	67	39 58.2		
Tonsils	75	45 60	25	16 64	100	61 61		

Unidentified group (b).

Morphology. Gram-negative coccobacilli, arranged singly, in two's and in clumps. Some strains were slightly pleomorphic, non-sporing, non-capsulated and non-motile both at 37°C. and 22°C. (Fig. 10.)

Blood agar plate. Round, convex, entire, smooth, greyish-white, easily emulsifiable colonies. No haemolysis.

Agar plate. Round, convex, opaque colonies.

Gelatin stab. No growth.

Broth. Profuse granular growth, slight flaky deposit, surface ring and scum.

Löffler's serum. Whitish or slightly yellowish raised growth. No liquefaction.

Potato. Slightly whitish growth.

MacConkey's agar plate. Profuse pale growth with yellowish zone.

Metabolism. Aerobic. No growth at all under strict anaerobic conditions. Optimum temperature 37°C. No growth at room temperature. Slow growth at 22°C.

Biochemical. Ferment only glucose with acid and no gas; Catalase positive; Indole negative; M.R. negative; V.P. negative; Nitrate reduction negative; NH₃ negative; H₂S negative; M.B. negative; L.M. no change. Urea was not decomposed into ammonia.

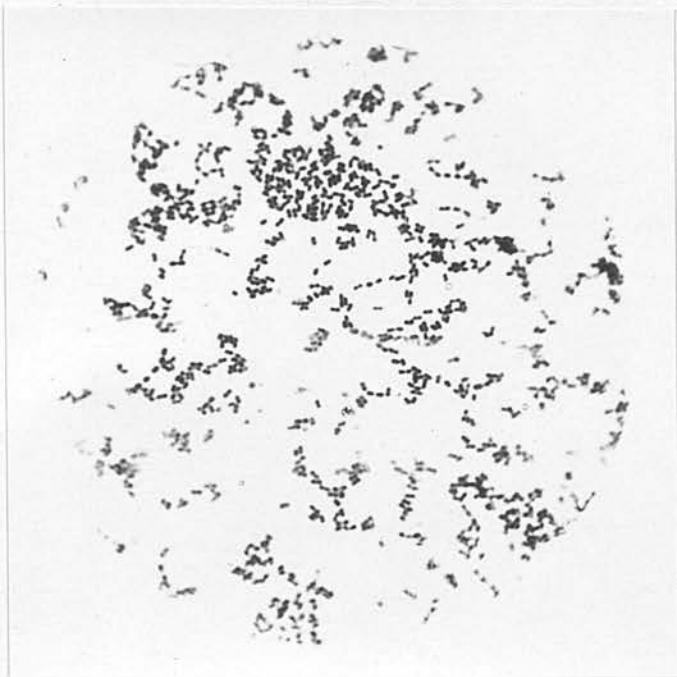


Fig. 10. - Gram-negative Coccobacilli
(unidentified group b)
From blood agar culture, 24 hours, 37°C.
(X 1200)

Pathogenicity. Non-pathogenic to mice, rabbits and guinea pigs.

Results. The percentage carrier rates for these organisms also were practically the same in normal and sick dogs (normal, 48 per cent.; sick, 44 per cent.). Their frequency in the nose, nasopharynx and tonsils of normal and sick dogs did not vary to any considerable degree. They were isolated from the tonsils of 37 per cent., from the nasopharynx of 32.8 per cent. and from the nose of only 6 per cent. of the dogs.

TABLE XVI

The frequency of unidentified group (b) in the nose, nasopharynx and tonsils

Situation	Normal Dogs		Sick Dogs		Combined	
	Number examined	Positive No. %	Number examined	Positive No. %	Number examined	Positive No. %
Nose	75	4 5.3	25	2 8	100	6 6
Nasopharynx	50	16 32	12	6 50.0	62	22 35.5
Tonsils	75	28 37.5	25	9 36	100	37 37

TABLE LV

Percentage carrier rates for unidentified group (b) in normal and sick dogs

Group	Number examined	Number Positive	Percent Positive
Normal	75	36	48
Sick	25	11	44
Combined	100	47	47

TABLE LVI

The frequency of unidentified group (b) in the nose, nasopharynx and tonsils

Situation	Normal Dogs		Sick Dogs		Combined	
	Number examined	Positive No. %	Number examined	Positive No. %	Number examined	Positive No. %
Nose	75	4 5.3	25	2 8	100	6 6
Nasopharynx	50	16 32	17	6 35.3	67	22 32.8
Tonsils	75	28 37.3	25	9 36	100	37 37

Unidentified group (c).

Morphology. Gram-negative small bacilli, 0.7 to 1.8 micron by 0.4 to 0.8 micron with straight axis and rounded ends, slightly pleomorphic, staining deeply at the ends, giving the appearance of bipolar staining, arranged singly, in pairs or in small bundles, not in chains; non-motile, non-sporing, non-capsulated and non-acid fast (Fig. 11).

Blood agar plate. Twenty-four hours at 37°C. Colonies were discrete, round, 0.5 to 1 mm. in diameter, low convex, amorphous, greyish-white, translucent with smooth glistening surface and entire edge; consistency butyrous and easily emulsifiable.

Agar plate. Colonies were the same as on blood agar, but smaller.

Gelatin stab. Two days at 22°C. Good filamentous growth, confluent at the top, discrete below, extending to the bottom. No liquefaction.

Broth. Twenty-four hours at 37°C. Moderate growth with moderate turbidity and a slight powdery or viscous deposit. No surface ring and no pellicle.

Löffler's serum. Good raised confluent whitish growth. No liquefaction.

Potato. No visible growth.

MacConkey agar plate. No growth.

Metabolism. Aerobe and facultative anaerobe. Growth under anaerobic conditions was rather poor. Slow growth at 22°C. Very poor growth at room temperature.

Biochemical/

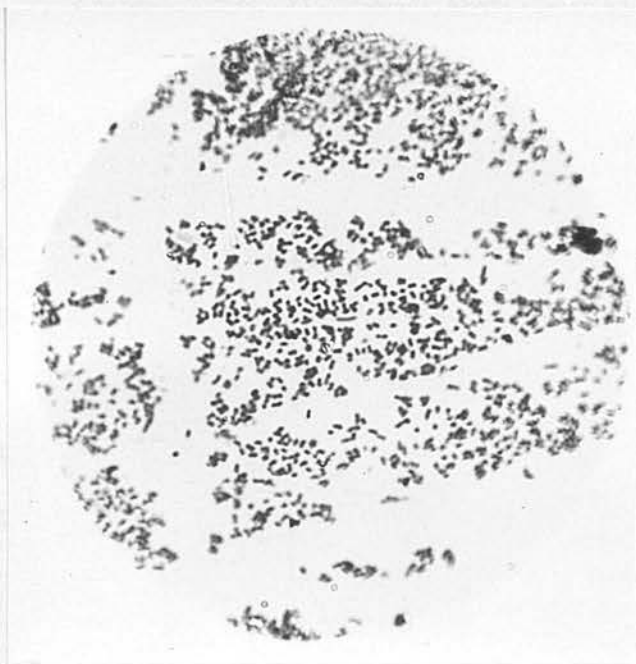


Fig. 11. - Gram-negative small bacilli
(unidentified group C).
From blood agar culture, 24 hours, 37°C.
(X 1200)

Biochemical. (Ref. Table No. LIX). There were only slight variations in the biochemical reactions of the various strains grouped under this head. All but eight strains formed indole, were positive for Catalase, only a few strains produced slight ammonia and H₂S. Most of them had no action on litmus milk, but a few changed it to slightly acid. Glucose was fermented by all the strains, maltose by all excepting two (4N_d and 4NP_d). Similarly, three strains (48T₆, 67NP₇ and 18 T_d) failed to ferment sucrose. Only three strains (3NP₁, 3 T₄, 10T₇) fermented inositol. Most of the strains fermented dextrose; trehalose was fermented by twenty strains and raffinose by only two strains (9NP₄ and 9T₄).

Pathogenicity. Non-pathogenic to mice, guinea pigs and rabbits.

Results. The percentage carrier rate for these organisms in normal dogs was 38, while it was slightly higher in the sick (40). They were more prevalent in the nasopharynx and tonsils of both normal and sick animals and were very rarely present in the nose of either group. Only one out of seventy-five normal dogs and two out of twenty-five sick dogs harboured them in the nose. But they were found in the nasopharynx of 30 per cent. of the normal and 29.4 per cent. of the sick animals, and also in the tonsils of 28 per cent. of the normal and 36 per cent. of the sick dogs.

TABLE LVII.

Percentage carrier rates for unidentified
(group(C) in normal and sick dogs.

Group	Number examined	Number Positive	Percent Positive
Normal	75	27	36
Sick	25	10	40
Combined	100	37	37

TABLE LVIII

Frequency of unidentified group (c) in the nose, nasopharynx
and tonsils

Situation	Normal Dogs		Sick Dogs		Combined	
	Number examined	Positive No. %	Number examined	Positive No. %	Number examined	Positive No. %
Nose	75	1 1.3	25	2 8	100	3 3
Nasopharynx	50	15 30	17	5 29.4	67	20 29.9
Tonsils	75	21 28	25	9 36	100	29 29

TABLE III

Biochemical reactions of unidentified group (c) isolated from normal and sick dogs.

Strain	Indole	M.P.	V.P.	NH ₃	H ₂ S	Nitrate	Catalase	M.B.	L.M.	Glucose	Lactose	Mannitol	Inositol	Maltose	Dulcitol	Sucrose	Salicin	Arabinose	Raffinose	Trehalose	Inulin	Dextrin	Rhamnose	Xylose	Sorbitol
1 T6	+	-	-	+ sl	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
3 NP1	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
3 T4	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
6 NP3	+	-	-	-	-	-	+	-	A sl.	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
8 NP9	+	-	-	-	-	-	+	-	A "	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
8 T8	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
9 NP4	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
9 T4	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
10 T7	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
12 T6	-	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
13 NP1	+	-	-	-	+ sl.	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
13 T2	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
14 T1	+	-	-	+ sl.	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
16 NP1	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
16 T2	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
17 NP1	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
19 T4	+	-	-	-	-	-	+	-	A sl.	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
20 NP3	+	-	-	-	-	-	+	-	A sl.	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
22 NP2	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
26 NP2	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
28 T4	+	-	-	+ sl.	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
31 T5	-	-	-	-	+ sl.	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
33 N3	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
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35 T2	+	-	-	-	-	-	+	-	A sl.	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
32 T8	+	-	-	-	+ sl.	-	+	-	A sl.	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-
42 NP4	+	-	-	-	-	-	+	-	-	A	-	-	-	A	-	A	-	-	-	-	-	A	-	-	-

TABLE LIX (Contd.)

Strain	Indole	M.R.	V.P.	NH ₃	H ₂ S	Nitrate	Catalase	M.B.	I.M.	Glucose	Lactose	Mannitol	Inositol	Maltose	Dulcitol	Sucrose	Salicin	Arabinose	Raffinose	Trehalose	Inulin	Dextrin	Thamnose	Xylose	Sorbitol
43 NP6	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
43 T6	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
45 T2	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
46 T7	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
48 NP5	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
48 T6	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
55 T4	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
63 T6	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
67 NP7	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
67 T5	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
1 NPF	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
1 TG	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
2 Te	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
3 NPc	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
3 Tf	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
4 Nd	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
4 NPD	-	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
4 Te	-	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
11 TG	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
14 Td	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
15 NPe	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
15 Th	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
18 Ne	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
18 Td	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
20 NPF	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
25 Td	+	-	-	-	-	-	+	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-

sl. = slight reaction, not distinct.

DISCUSSION.

This investigation presents clear data of the presence of the various bacterial species, both those regarded as potential pathogens and those believed to be saprophytes, in the upper respiratory tracts of presumably normal dogs and the variations met with in sick dogs.

Amongst the pathogens were found to be included Staphylococci (present in 70.7% of the normal and 84% of the sick dogs), haemolytic streptococci (normal, 64%; sick, 68%), *H. bronchisepticus* (normal, 18.7%; sick, 52%) and Friedländer's bacillus (normal, nil; sick, 16%). Amongst the facultatively pathogenic were non-haemolytic streptococci (normal, 77.3%; sick, 96%), Coliform bacilli (normal, 21.3%; sick, 48%), *Bacterium alkaligenes* (normal, 36%; sick, 60%), and *Proteus* sp. (normal, 16%; sick, 28%), whilst the remaining organisms, namely, Gram-negative cocci (normal, 60%; sick, 56%), diphtheroid bacilli (normal, 18.7%; sick, 28%), *B. subtilis* group (normal, 16%; sick, 20%), *H. canis* (normal, 12%; sick, 8%), *Leptotrichia* sp. (normal, 24%; sick, 28%), and unidentified Gram-negative bacilli groups a (normal, 60%; sick, 64%), b (normal, 48%; sick, 44%), and c (normal, 36%; sick, 40%) were all regarded as saprophytes.

Staphylococci: Accepting coagulase production as the criterion of pathogenicity of staphylococci of animal origin as is adopted for human strains, the frequency of pathogenic strains (49.3 per cent.) found in the upper respiratory tracts of normal dogs in this study is surprisingly high. No information of the kind has so far been available and no consideration has been given to the part these organisms might play in any of the diseases of dogs. In addition to Coagulase production, the majority of these strains also satisfy other tests of pathogenicity, such as liquefaction of solid serum and production of haemolysins. Moreover, all the Coagulase-positive strains isolated in this study compare very favourably with the pathogenic strains isolated and studied by Smith (1947) from acute infections in dogs or from lesions in the same animals in which the organisms were believed to be responsible for the pathological changes. Further, it was observed that the frequencies of pathogenic strains in all the situations (nose, nasopharynx and tonsils) were considerably higher (68 per cent.) in sick dogs than in normal dogs (49.3 per cent.) These factors lead one to the conclusion that staphylococci have some part to play either primarily or secondarily in the respiratory or other diseases of dogs and that these organisms may not be of lesser importance than beta-haemolytic Streptococci which have recently gained so much prominence as the causative agents/

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agents of infections in dogs. The same view was held by Miss M. Freak, who during the course of the discussion on Dr Tom Hare's paper (1946) on "Beta haemolytic Streptococcus infection in dogs", stated that haemolytic staphylococci had been found in a high percentage of swabs from suspected streptococcal infections of all types (mostly recurrent tonsillitis or respiratory cases and some in skin diseases too). She also quoted a case of a young spaniel bitch showing skin lesions and slight inflammation of the tonsils and pharynx with slight pyrexia, which yielded haemolytic Staphylococci, both from the tonsils and skin lesions. Bosworth (1947) observed that Staphylococci were not so common in tonsil swabs as Streptococci, but he reported that they were occasionally met with in cases of swollen throat and cervical lymphatic glands and also in furunculosis. He also agreed that no adequate survey of the incidence of staphylococci in dogs in health and disease had yet been made. Bosworth nor any other previous worker does not seem to have examined the nasal swabs from dogs, which would no doubt have shown a high frequency of staphylococci in that situation, possibly higher than the frequency of haemolytic Streptococci in the tonsils of the same animals, as has been found in this study. Probably the failure to examine nasal swabs accounts for the fact that these organisms were not considered so/

so prevalent in dogs and no detailed study of them has so far been made. But the data presented in this investigation should offer ample justification to study the association of these organisms with various diseases of dogs, and it may be suggested that in every case the pathogenicity of the strain should be ascertained by its ability to produce Coagulase, as other tests have been proved not reliable.

Haemolytic streptococci: Although the isolation of beta haemolytic Streptococci from the tonsils of apparently normal dogs and from cases of tonsillitis and pharyngitis have been reported by some workers, any information about their presence and frequency in the nose and nasopharynx has been completely lacking up to the present, though their presence in these situations has been assumed. Further, the distribution of different serological groups even in the tonsils has not so far been properly surveyed. Pilot et al (1936) did not group their strains. Garside (1947) grouped only a few of the strains which he isolated from the tonsils of 200 normal dogs in his two surveys, and most of them belonged to group G. But it was recorded by Garside that 82 per cent. of the total strains isolated from different situations in dogs, mostly from infective processes, belonged to group G. Similarly, Hare and Fry (1938b) observed that/

that 61 per cent. of the total strains isolated from various conditions in dogs belonged to group G.

Though the percentage (48.2 per cent.) of group G strains isolated in this investigation fell short of the above figures, it still remained the predominant group. It is interesting that such high percentages as 25.7 per cent. of group M and 10.5 per cent. of group L were isolated. Such figures have not so far been recorded and may be due to accident in that their presence may not be a constant feature but subject to variation or it may be that previous workers were handicapped for want of a good supply of L and M grouping sera.

The frequency of group M strains in sick dogs (12 per cent.) was decidedly less than their frequency in normal dogs (20 per cent.), while all the remaining groups, except group F, showed higher frequencies in sick dogs, as shown in Table XVII. Group F was found in only two normal dogs. So these two, group M and group F, appear to have no pathogenic significance in these animals, although further investigation may reveal the contrary.

The isolation of a group A strain from the tonsils of dog No. 12 and group C from the nose of the same dog, and also group C from the nose of dog No. 7, while the tonsils and nasopharynx of the same animal showed group B strains, indicates the possibility of the co-existence of more than one serological group in/
in/

in the same situation as well as in one animal. Such instances may be frequent, but in the present investigation since in order to conserve media only one colony of haemolytic streptococci was picked up from each of the original plates, there was no chance of meeting two different strains in one situation, even if they were present.

Another point of interest is that while the positive carrier rate in the nose of normal dogs was 13.3 per cent., it rose to 32 per cent. in sick dogs, while the difference in the nasopharynx and tonsils of the two groups of dogs was not very marked. This increase might have been due to the extension of these organisms from the nasopharynx to the nasal cavities owing to the catarrhal conditions of the upper respiratory tracts, as most of the sick dogs studied had nasal catarrh and discharge.

Another question arising from the results of this investigation is whether the claims made by Hare (1946) can be upheld. Hare maintains that beta haemolytic Streptococci are never found in normal dogs and that when they are isolated from supposedly normal dogs it indicates a cyclical waning of a chronic infection. But the present investigation shows that forty-eight (64 per cent.) of the seventy-five apparently normal dogs harboured haemolytic Streptococci, and it does not/

not seem possible that such a high percentage of dogs which appeared perfectly healthy at the time of swabbing could have been in the waning phase of the infection. In any case, where there is no sign of even chronic infection, the animal has to be classed as a carrier until the indicative signs of the chronic infection are recorded.

Such a high percentage of carriers among the dog population does support the observations of the importance of haemolytic streptococci as pathogens of dogs, for transmission of these organisms to susceptible animals by licking, through the agency of common feeding troughs or by droplet infection, is easily accomplished. It is also an easy possibility that new-born puppies contract the infection from their own dam either from infected vaginal exudate during parturition or by post-natal licking, as suggested by Hare.

Isolations of groups C, G and A from normal and sick dogs indicate the possibility of cross-infection between dog and man, as groups C and G are not infrequently found in human infections, and group A is a proved human pathogen.

Haemophilus bronchisepticus: This organism, which was once considered to be the main aetiological factor in distemper, has now lost its importance as such, but it is still regarded as responsible for the/

the respiratory complications in distemper. The claims made by Ferry, McGowan, Torrey and Rahe that this organism was recoverable from a very high percentage of cases in the initial stages of distemper are amply confirmed by the results of this investigation, wherein the organism was recovered from the majority of the dogs showing clinical symptoms of distemper.

Another point of interest is that while previous workers did not hold the view that this organism was recoverable from healthy dogs, it was isolated from a small percentage (18.7 per cent.) of the apparently normal dogs in this investigation. Such a low carrier rate amongst normal dogs and its presence in such a high percentage of sick dogs imply its constant association with this disease.

It is also believed that this organism is capable of causing respiratory diseases, such as contagious pneumonia in dogs, independent of the distemper virus (Wooldridge, 1934).

It is now known that the antigen and the toxin of this organism are related to the corresponding substances in *H. pertussis* and *H. parapertussis*. Further there are also references in medical literature to conditions caused by *H. bronchisepticus* in human beings which simulated whooping cough (Brown, 1926) and the common cold (Walker, 1928). MacGowan (1911) also recorded the isolation of this organism from the nose of a laboratory worker showing symptoms of chronic nasal catarrh. But there has been no reference/

reference in recent years of any human infection with this organism. However, on the basis of the recorded evidences, it may be safe to assume that it is mildly pathogenic to human beings also.

Friedländer's bacillus: It is rather difficult to distinguish Friedländer's bacillus from the coli-aerogenes group as the latter also show a small capsule, and also no marked differences exist in the cultural characteristics and biochemical reactions of the two groups. It is generally considered that Bact. aerogenes is a saprophyte of grains, the intermediate type of Coliform bacillus is a saprophyte of soil and the Friedländer's bacillus is the parasite of the respiratory tract of man and animals. But often it is found that most Friedländer's strains of respiratory origin in human beings are indistinguishable from the B. Coli intermediate type and many strains found in cystitis are identical with Bact. aerogenes, so it is not possible to differentiate these organisms on the basis of habitat alone.

In this study, while assigning all the atypical strains of B. Coli isolated from normal and sick dogs to the Coliform bacilli, five strains isolated from sick dogs were singled out as belonging to Friedländer's group on account of their characteristic nail-headed appearance in gelatin stab culture and of their mild pathogenicity to mice. Perhaps all/

all, or possibly some, of the atypical groups of B. Coli also, isolated from normal and sick dogs may have belonged to Friedländer's pneumobacillus group antigenically.

Friedländer's bacillus in human beings is recorded as associated with catarrhal conditions of the respiratory tract, suppuration in the nasal sinuses, meningitis, conjunctivitis and cystitis. References are also available of its isolation from animal sources. Edwards (1928) isolated Friedländer's bacillus from metritis of mares, while Webster (1928, 1930) described a respiratory epidemic in mice caused by this organism. Wallace, Cahn and Thomas (1933) found it in a paralytic disease of moose, while Brion and Lucan (1941) isolated an organism resembling Friedländer's bacillus from the brain culture of two dogs which had died showing spastic paraplegia, with absence of Achilles tendon and plantar reflexes and of reflex reaction on the posterior part of the abdominal wall. Gray (1942) isolated this organism from a case of cystitis in a dog. Although no more references are available of the isolation of Friedländer's bacillus from normal or sick dogs, it may reasonably be assumed that the organism is capable of exhibiting pathogenic properties on occasion.

Non-haemolytic Streptococci: Non-haemolytic streptococci of the throat of any species of animal have not so far been studied. Even in the medical field these organisms still remain ill-defined, and they are usually referred to as streptococci of the "Salivary group", as "Streptococcus viridans" and "indifferent streptococci", according to the degree of greening produced on blood agar, or simply as the "mouth streptococci".

In their early classical work, Andrews and Horder (1906) described the predominant Streptococci of the human throat as *Str. salivarius* and *Str. mitis*, but since they were not able to draw a clear distinction between these two species, they considered that *Str. mitis* was a variant type of *Str. salivarius*. Safford, Sherman and Hodge (1937) also did not find justification for rigidly differentiating these two species, while in the compilations of Sherman (1937) both species are considered to be *Str. salivarius*, which is a heterogeneous group. Subsequently, although Niven, Smiley and Sherman (1941a, 1941b) and Sherman, Niven and Smiley (1943) differentiated *Str. salivarius* from *Str. mitis* by a few physiological characters, one may still consider that the difference is not so marked as to warrant separate names for them. For the purpose of this study, however, all the strains showing the biochemical characteristics of "Salivarius" and "mitis" as described by Sherman et al (1943) were classified as *Str. salivarius*.

Lactose non-fermenting strains were considered as *Str. equinus* (Sherman, 1937). This organism, which is predominant in the intestine of the horse, is found also in bovine and human faeces (Winslow and Palmer, 1910). It has also been frequently obtained from the human throat. Since it also shows points of close relationship with *Str. salivarius*, Sherman and his co-workers consider that it is one of the variant types of *Str. salivarius*.

Streptococcus bovis was first described by Winslow and Palmer (loc. cit.) as the prevailing type of streptococcus of the bovine species, though Fuller and Armstrong (1913) considered this organism to be identical with *Str. salivarius* of the human throat. Orla-Jensen (1919) described it as a new species on account of its fermentative properties, especially its reaction on arabinose. Ayers and Mudge (1923) found these organisms predominantly in the mouth and intestines of cows, and again, Sherman et al consider these organisms also to be variants of *Str. salivarius*.

Streptococcus faecalis was first described by Andrews and Horder (loc. cit.). In addition to being the predominant streptococcus in the human intestines, it is probable that it is found also in animals. The fact that some animals, notably the horse and the cow, harbour other types which make up the prevailing streptococcal flora has led to statements which imply that *Str. faecalis* is peculiar to the human intestinal tract/

tract, but a number of investigators have reported this organism as occurring in the faeces of cattle, horses and other domestic animals. Although *Str. faecalis* is not classed as a pathogenic organism, it has been known since the original description of Andrews and Horder to occur occasionally in cases of endocarditis and other human infections.

It is interesting that strains belonging to all the groups referred to above were isolated from the upper respiratory tracts of dogs. *Str. salivarius*, which is the predominant organism of the human throat, seems to be a normal inhabitant of the throats of dogs also, since they were so frequently found in these animals. 64.7 per cent. of non-haemolytic streptococci isolated from normal and sick dogs belonged to this group. Other groups were *Str. faecalis* (12.2 per cent.), *Str. equinus* (12.8 per cent.) and *Str. bovis* (6.8 per cent.). These organisms which are considered to be the inhabitants of the intestinal tracts of man and animals are likely to gain access into the nasopharynx of dogs on account of the constant habit of these animals of licking and sniffing faecal materials.

As with the haemolytic streptococci, the frequency of these organisms was the highest in the tonsils (68 per cent.), less in the nasopharynx (47.8 per cent.) and lowest in the nose (33 per cent.) in both normal and sick dogs.

What part these organisms play in health and disease in dogs is beyond the knowledge of any veterinary pathologist at present, and much more research will be necessary before arriving at any conclusion.

However, the greater frequency of these organisms in all situations in sick dogs (96 per cent.) than in normal dogs (77.3 per cent.) possibly indicates that they play some part in diseases in these animals. It may also be stressed that the biochemical characteristics of many of the strains isolated in dogs are similar to those isolated from human throats, indicating the possibility of cross-infection between man and animal.

Coliform bacilli: Although these organisms are generally regarded as non-pathogenic under ordinary conditions, their pathogenicity in man and animals under favourable circumstances have been recorded. In human beings they are frequently found in pyogenic infections of the urinary tract (pyelitis, cystitis, etc.) either in pure culture or mixed with pyogenic cocci, and also in Cholecystitis, cholangitis, appendix abscess, peritonitis and septic wounds invariably mixed with other pyogenic organisms (Mackie and McCartney, 1948b).

In animals, organisms of the B. coli type are generally regarded as causative agents of the disease of young calves (particularly those deprived of the colostrum/

colostrum of dams) commonly called "white scours" or "calf scours" (Smith and Little, 1922; Smith and Orcutt, 1925). According to Gwatkin, Legard and Hadwen (1938) and Minett, Stableforth and Edwards (1929), bovine mastitis also may occasionally be due to Coliform bacilli. Howarth (1932) has reported a large outbreak of abortion in sheep in California in which an organism belonging to the colon group seemed to be the causative agent. But the presence of *B. coli* in the upper respiratory tracts of dogs or their association in any of the diseases of dogs has not so far been reported. It is presumed that Coliform organisms play a secondary role in the complications of distemper, but no authentic information substantiating this view is yet available.

In this investigation, organisms belonging to typical and atypical types of *B. coli* were encountered in 21.3 per cent. of the normal and 48 per cent. of the sick dogs. However difficult it may be to assess their pathogenic significance, yet taking into account the few reports referred to above indicating their pathogenicity in man and some species of animals, it may be assumed that Coliform bacilli may associate with other organisms in the complications, particularly respiratory or gastric, of distemper.

Bacterium alkaligenes: No study of this organism in domestic animals has yet been made. In point of fact, even in the medical field it still remains inadequately/

inadequately studied. Petruschky (1896) isolated this organism from human faeces and named it "Bacterium faecalis alkaligenes". Again, Nyberg (1935) studied a large number of strains and found two distinct forms and a number of well differentiated types. They included motile and non-motile strains producing marked alkalinity or no change at all in litmus milk. None of the strains had any action on sugars. All the strains studied by Nyberg also had their origin in human excreta, but it is believed that though these organisms are the constant inhabitants of the intestinal tract of man, they are also found in decaying materials, dairy products and soil. Since no study of the antigenic structure of these organisms has so far been made, it is not known whether or not the strains of faecal origin and from other sources are antigenically similar. Though they are generally regarded as non-pathogenic, they may occasionally give rise to infections of the enteric type (Petruschky, 1896; Hirst, 1917; Khaled, 1923). They are often found as one of the commonest concomitants in human dysentery, and cases of *B. faecalis alkaligenes* bacteraemia have also been recorded (Mackie and MacCartney, 1948c).

The significance of these organisms in the upper respiratory tracts of dogs and the part they may play in respiratory affections are not known.

But/

But their increased frequencies in sick dogs lead one to believe that they may have some role in the respiratory complications of distemper, however minor that may be.

Proteus species: The occurrence and pathogenicity of this species of organism in human beings is fairly well known. They are frequently found in, and stated to be responsible for, some of the inflammatory and suppurative conditions in man, are said to be the common cause of cystitis and are isolated in pure culture from the urine of infected patients. They are also found in abscesses, either alone or in combination with other organisms. These organisms have also been isolated from a variety of conditions, such as Volvulus, peritonitis, pneumonia, acute gastro-enteritis of the food-poisoning type, empyema, lung gangrene and septicaemia (Wichels and Barner, 1925; Plahn, 1937; Cooper et al, 1941). Besides, many strains referred to as Proteus strains have been isolated from the urine, faeces or blood of patients suffering from typhus fever, though the exact relationship to the aetiological agent of this disease is still obscure.

In contrast to human medicine, very little study of this group of organisms has so far been done in any species of animals. Jensen (1913) considered them to be responsible for one form of epidemic calf dysentery, and Wyss (1898) has isolated them in an epidemic disease of fish. They are also reported as responsible/

responsible for black rot of eggs (Miles, 1937). Beyond this no more information regarding the isolation of these organisms from any species of animal, either in health or in disease, is available. Taking into account the association of these organisms with a variety of conditions, particularly inflammatory and suppurative in man, it may be presumed that the presence of them in the upper respiratory tracts of dogs may not be without pathogenic significance. They may play a role as secondary invaders in the respiratory diseases of dogs, even though they may not be primarily responsible for such conditions. Further, since distemper in dogs is mostly characterised by catarrhal conditions of the respiratory tract and often of the alimentary tract too, these organisms may obviously be active in precipitating respiratory or gastric complications in that disease, either independently or associated with other secondary invaders.

Neisseria: Practically no study has so far been made regarding the Gram-negative cocci from animals, and no reference is available of the isolation of these organisms from any species of animal.

Sufficient study has been made from time to time of the common Gram-negative cocci in the nasopharynx of human beings. Pathogenic significance has in general been attributed to the meningococcus, but *N. catarrhalis*/

N. catarrhalis is not considered to be an absolutely harmless parasite. Pfeiffer (1896) described the Gram-negative coccus which is now known as *N. catarrhalis*. This organism was subsequently studied by Ghon and Pfeiffer (1902), who isolated it from cases of acute bronchitis. Gordon (1921) examined Gram-negative cocci from the nose and throat of normal persons and of persons with colds or influenza.

On the basis of carbohydrate reactions, he classified them into several groups, but out of 246 of his strains, 103 fell into *N. catarrhalis* and a small percentage to *N. pharyngis*. Dochez et al (1929) found Gram-negative cocci in 99 per cent. of the nasopharyngeal cultures and in one per cent. of the nasal cultures. Gundel and Linden (1931) found Gram-negative cocci in 100 per cent. of school children. Burkley and Smillie (1929) found that nearly all the healthy people they swabbed carried Gram-negative cocci. Straker et al (1939) found them in 90 per cent. of the individuals in London and South-east England. None of these workers discussed the pathogenic significance of these Gram-negative cocci present in the nasopharynx of normal human beings.

Neisseria catarrhalis I and *Neisseria pharyngis* isolated from the upper respiratory tracts of dogs compare very favourably with the "catarrhalis" and "pharyngis" group isolated from the human nasopharynx both culturally and biochemically, but *N. catarrhalis* /

N. catarrhalis II seems to be quite a new type which has not been isolated and described so far even from the human nasopharynx. But an organism described by Huntoon (1934) as a probable new member of the genus *Neisseria* somewhat resembles this latter organism in its cultural aspects. Huntoon isolated a Gram-negative coccus from human lung sputum in two cases. It resembled *N. catarrhalis* in its inability to ferment any sugar, but culturally it more nearly resembled *N. meningitidis* in being very delicate, dying out quickly in artificial media and in its inability to grow at room temperature. Furthermore, in contradiction to *N. catarrhalis*, it formed a perfect emulsion in salt solution. Since this organism did not correspond to any of the described members of the group, Huntoon considered it to be a new species and proposed the name "*Neisseria pseudocatarrhalis*". Unfortunately, a detailed description of this organism regarding its morphology and cultural characteristics is lacking. So it is not possible to say definitely whether *Neisseria Catarrhalis* II, isolated in this investigation is the same organism, but there is some similarity when the recorded cultural characteristics are compared.

It was not possible to make a very detailed study of the Gram-negative cocci isolated in this investigation and to establish the taxonomic relationship of different types and also their pathogenicity, but/

but the frequency of occurrence in both normal and sick dogs suggests that further investigation would not be unprofitable.

Diphtheroid bacilli: Only two species of diphtheroid bacilli, namely *C. hofmanni* and *C. xerosis*, were met with both in normal and sick dogs. The pseudo-diphtheria or Hofmann's bacillus is an organism very frequently found in the throats and noses of human beings. Similarly, *C. xerosis* is the commonest bacterial inhabitant of the normal conjunctival sac of human beings. Both are well recognised species among those non-pathogenic to man. The isolation of these or any other species within this group from the upper respiratory tract of dogs has not yet been reported, but organisms closely resembling *C. xerosis* morphologically, culturally and biochemically were isolated by Smith (1904) from the normal conjunctival sac of dogs and were named *C. xerosis canis*. Since both these species in human beings are believed to be non-pathogenic and furthermore no information is available of any disease in dogs caused by Diphtheroids, it may be presumed that these organisms enjoy a purely saprophytic existence in the upper respiratory tract of these animals.

Bacillus subtilis group: These organisms are generally considered as saprophytes found in hay, soil, water and dust. Being ubiquitous they are found to be the common contaminants of culture media in/

in the laboratory. It may be presumed that the isolation of these organisms from the nasal swabs of quite a small percentage of normal and sick dogs (normal, 16%; sick, 20%) was quite accidental, the organism having gained access to the nasal mucosa as a result of inhalations of dust containing the spores and probably they are not the natural inhabitants of these parts. The fact that the tonsils and nasopharynx were comparatively free of these organisms supports this conclusion.

Haemophilus canis: Although the isolation of this organism from the respiratory tract of dogs has not been previously reported, it was, however, isolated by Friedberger (1903) and Rivers (1922) from the preputial secretions of dogs. Friedberger named his organism Haemoglobinophilus canis in view of its haemoglobin requirements for growth. From his description, it is difficult to see how he differentiated it from Haemophilus influenzae, except that he isolated it from dogs instead of from human beings. Odaira (1911) compared Haemophilus pertussis H. influenzae and H. canis and found by agglutination tests that they were different. From a detailed study of its cultural and biochemical characteristics, Rivers found this organism differed from H. pertussis by indol production, nitrate reduction and sugar fermentation and from H. influenzae by its in-
difference/

difference to one of the accessory growth factors and mannitol fermentation.

According to previous workers, this organism is found in predominant numbers in the preputial secretions of dogs, and so it is a matter of doubt whether its presence in the upper respiratory tract of a small proportion (11 per cent.) of dogs is only accidental, due to the organism gaining access to those parts as a result of the common habit of dogs licking their preputial secretions, or whether it is also a natural inhabitant of the respiratory tract. But, in view of the fact that out of the eleven dogs from which the organism was recovered five were females, in which the chances of infection from the preputial secretions were remote, one is inclined to believe that *H. canis* has a parasitic existence in the upper respiratory tract (in all situations) of a small percentage of dogs, though it is predominantly an organism of the preputial secretions.

This organism has not been reported in any other species of animal, but an organism of similar morphology and cultural characteristics, requiring only "X" factor for its growth was reported to have been isolated on one occasion only by Fleming (1930) from the human mouth. Similarly, seven out of eighty strains of bacilli belonging to the "haemophilus group" isolated by Milne (1934) from human tonsils needed only/

only "X" factor. Neither of these authors studied the biochemical reactions of their strains, but in view of their nutritional requirements, it was thought that they belonged to the "H. canis" group. Beyond these, no other reference is available to the isolation of this organism from human sources.

Leptotrichia species: No reference to the isolation of such filamentous organisms from the respiratory tracts of any species of animals or, as a matter of fact, from any animal source, is available, nor is much information available regarding the isolation of such organisms from human sources, but the work of Bibby and Berry (1939) on "A cultural study of Filamentous bacteria obtained from the human mouth" throws some light on the problem. These authors isolated several strains of filamentous bacteria from aerobic and anaerobic cultures made from scrapings from the teeth, gingivae, and mucous surface at various sites in healthy and diseased mouths of adults and children and classified them into several groups on the basis of their morphology and colonial forms. Bibby and Berry thought that the majority of the strains had the characteristics of the genus *Leptotrichia* according to Bergey's definition and so they suggested the name, *Leptotrichia buccalis* for these strains, even though they were not quite identical in their morphology and cultural characteristics with *Leptotrichia buccalis* (Robin) Trevisan described by Bergey.

The strains isolated in this study from the tonsils and nasopharynges of dogs closely resembled some of the strains of Bibby and Berry in their morphology and also in their staining and cultural reactions and, so, while definitely assigning them to the genus, *Leptotrichia*, the specific name *Leptotrichia buccalis* may be suggested for these filamentous organisms also.

The isolation of these organisms in this investigation from the tonsils and nasopharynges of dogs, indicates the possibility of establishing the existence of some more types of filamentous organisms in these situations and also in the mouths of dogs by a thorough investigation, using various culture media and also anaerobic methods.

Unidentified group (a): These organisms which were recovered from 60 per cent. of the normal and 64 percent. of the sick dogs, most frequently from the tonsils and nasopharynx and occasionally from the nose, could not be definitely identified as they did not correspond with any described organism. Their characteristics most nearly resemble the genus *Flavobacterium* and the nearest related species within that genus appears to be *Flavobacterium fecale* (Bergey et al, 1939) in view of the liquefaction of gelatin and solid serum, the peptonization of milk and the absence of fermentation of any of the carbohydrates. Indole production has not been recorded in this species.

According/

According to the situations from which these organisms were frequently isolated, one would have expected them to belong to the species, *Flavobacterium buccalis*, but the cultural characteristics agree more closely with *F. fecale*. However, taking into account the peculiar habit in dogs of licking and sniffing faecal materials and genitalia, the chances of finding organisms of faecal origin in the nasopharynx are not remote.

Since all the strains were uniform in their morphology, cultural characteristics and biochemical reactions it may be assumed that they all belonged to a single species, probably of saprophytic habits.

Unidentified group (b): These organisms were described as Gram-negative non-motile coccobacilli growing profusely on ordinary media, including MacConkey's agar, having action only on glucose (acid without gas), isolated from 48 per cent. of the normal and 44 per cent. of the sick dogs, also could not be identified definitely. Perez (1901, 1913) isolated from the nasal mucosa and saliva of normal and sick dogs a small coccobacillus fermenting glucose only with acid and gas and producing indole. But in the case of the organisms isolated in this investigation no gas was formed in glucose and indole was not produced. So they do not appear to be Perez's bacillus. It is said that certain strains of proteus ferment only glucose with acid, are non-motile/

motile and do not spread on solid media (Nelson, 1947) but these organisms did not decompose urea into ammonia, which is believed to be the important characteristic of proteus organisms (Rustigan and Stuart, 1945), thus they do not find a place with proteus. Miles (1937) has described a small Gram-negative coccobacillus, fermenting only glucose with acid, associated with black rot in eggs, but he too neither identified his organism nor named it. However, taking the morphology and cultural characteristics into consideration, these organisms may be grouped among the genus bacterium, although the probable species could not be ascertained.

Unidentified group (c): This group of organisms also could not be identified as by their cultural and biochemical characteristics they do not fit into any of the known species of micro-organisms.

Two probable genera to which they may belong are Actinobacillus or Pasteurella. Although they agree with the former, to some extent by growth characteristics and biochemical reactions, animal inoculation tests failed to confirm them as such. Intra-peritoneal inoculations of the cultures to male guinea pigs failed to produce either Strauss reaction or any other appreciable pathological changes.

Considering the situations from which these organisms were isolated, it is more probable that they belong to the Pasteurella species, which are the parasites/

parasites of the respiratory tract of animals and birds. They agree with *Pasteurella* as far as their cultural characteristics are concerned, with discrete colonies without very profuse growth on nutrient agar and absence of growth on bile containing medium. Further, biochemically - the formation of indole by most of the strains, the absence of gas in sugar media and the absence of any appreciable reaction in litmus milk are indicative of *Pasteurella* species, even though the fermentation reactions do not fully correspond with those of typical *Pasteurella*.

Atypical strains of *Pasteurella* species, non-pathogenic to laboratory animals, showing somewhat similar cultural and biochemical characteristics have been described by Jones (1921), Tweed and Eddington (1930), Newson and Cross (1932) and Rosenbusch and Merchant (1939).

In view of these facts, the strains described under this group may provisionally be regarded as atypical (non-pathogenic) *Pasteurella* organisms.

The isolation of the *Pasteurella septica* strains from dog bite wounds with profuse painful swelling and discharge attended with axillary adenitis in three human cases was reported by Allott et al (1944) and so particular care was taken during this investigation to see whether or not *Pasteurella septica* were present in the throats of dogs. But it is/

is clear from the results that no such typical and pathogenic strain was isolated even on a single occasion, whereas strains considered to be atypical and non-virulent at the time of isolation were found fairly frequently. In one of the cases studied by Allott et al a typical virulent strain of *Past. septica* was isolated in pure culture from the wound, whereas from the mouth of the dog, which had caused the wound by its bite, only a *Pasteurella*-like organism biologically atypical and non-pathogenic for laboratory animals was found. Since the mouths and throats of other two dogs were not studied, it is not known whether they harboured typical or atypical strains of this organism at the time of the bite. However, the isolation of a biologically typical and virulent strain from the wound and an atypical strain from the mouth of the dog should attract one's imagination as to the possibility of the atypical strains changing their biological characters and acquiring virulence when injected into human tissues.

In the absence of adequate data regarding the microbial flora of the respiratory tracts of dogs, pulmonary complications of distemper have hitherto been attributed only to *H. bronchisepticus*, but since this study has shown that there are other pathogens also present in the nasopharynx of normal and/

and sick dogs, there is the possibility that other organisms may also be responsible for such complications, either independently or in association among themselves or with *H. bronchisepticus*. Haemolytic streptococci, Coagulase-positive staphylococci and Friedländer's bacillus, which are potential pathogens may have an active role in this phenomenon, while non-haemolytic streptococci, Coliform bacilli, *bacterium alkaligenes* and *proteus* sp. which are believed to be mildly pathogenic may also have some minor part to play by way of complications or in aggravating any of the symptoms. The series of syndromes in dogs described by Hare, said to be caused by infection with beta-haemolytic streptococci, might also have been complicated by one or more of these organisms. Hare seems to have looked only for haemolytic streptococci and did not enquire for the presence of any other organisms that might have been in association with the streptococci in causing either all or some of the disease syndromes described by him.

Regarding other organisms, namely, Gram-negative cocci, diphtheroid bacilli, anthracoides, *H. canis*, *Leptotrichia* sp. and unidentified Gram-negative bacilli, it is difficult to indicate whether they have only a saprophytic existence in those situations or if they are capable of exercising any degree of pathogenicity under favourable conditions.

Finally, it may be emphasized that the important human pathogens, naemly *Str. pneumoniae*, *H. influenzae*, *N. meningitidis* and *C. diphtheriae*, which are normal inhabitants of the human nasopharynx were not found in the nasopharynx of dogs. *micro-organisms in the nose, nasopharynx and tonsils, and as per the morphology, cultural and biochemical characteristics, these organisms were classified into the following fourteen main groups and studied in detail.*

- a. *Staphylococci*
- b. *Haemolytic Streptococci.*
- c. *Neisseria bronchiseptica.*
- d. *Bacillus of Friedlander.*
- e. *Non-haemolytic Streptococci.*
- f. *Coli-form bacilli.*
- g. *Bacterium alkaligenae.*
- h. *Proteus sp.*
- i. *Gram-negative cocci.*
- j. *Diphtheric bacilli.*
- k. *Bacillus subtilis group.*
- l. *Neisseria catarrhalis.*
- m. *Leptotrichia sp.*
- n. *Unidentified Gram-negative bacilli.*

The incidence of these organisms in normal and sick dogs and their frequency in different situations (nose, nasopharynx and tonsils) were recorded.

SUMMARY.

1. One hundred presumably normal dogs and twenty-five sick dogs, all obtained from the outpatients' clinic of the Royal (Dick) Veterinary College, were studied for the presence of various micro-organisms in the nose, nasopharynx and tonsils, and as per the morphology, cultural and biochemical characteristics, these organisms were classified into the following fourteen main groups and studied in detail.

- a. Staphylococci
- b. Haemolytic streptococci.
- c. Haemophilus bronchisepticus.
- d. Bacillus of Friedländer.
- e. Non-haemolytic Streptococci.
- f. Coliform bacilli.
- g. Bacterium alkaligenes.
- h. Proteus sp.
- i. Gram-negative cocci.
- j. Diphtheroid bacilli.
- k. Bacillus subtilis group.
- l. Haemophilus canis.
- m. Leptotrichia sp.
- n. Unidentified Gram-negative bacilli.

2. The incidence of these organisms in normal and sick dogs and their frequencies in different situations (nose, nasopharynx and tonsils) were recorded.

3. Staphylococci: These were more frequently found in the nose, less in the nasopharynx and still less in the tonsils of both normal and sick dogs. 70.7 per cent. of the apparently normal and 64 per cent. of the sick animals were harbouring these organisms in one or more situations, while 49.3 per cent. of the normal and 68 per cent. of the sick yielded pathogenic (coagulase positive) strains.

4. Haemolytic streptococci: 64 per cent. of the normal and 68 per cent. of the sick dogs were the carriers of these organisms. The average percentage carrier rates in the nose, nasopharynx and tonsils were 18, 47.7 and 64 respectively. 48.2 per cent. of the strains belonged to Lancefield's serological group G, 13.2 per cent. to C., 23.7 per cent. to M, 10.5 per cent. to L., 2.6 per cent. to A and 1.8 per cent. to F. Group G. was lower, whereas groups M and L were higher than the figures recorded by previous workers. The public health aspect of the occurrence of groups G, C and A was duly stressed.

5. Haemophilus bronchisepticus: This was isolated from 18.7 per cent. of the normal and 52 per cent. of the sick dogs. Amongst the normal, mostly young animals were found to be carriers. The strains isolated in this investigation were typical of those described by previous workers regarding cultural and biochemical characteristics and also pathogenicity tests. Human infections with this organism were also quoted.

6. Friedländer's bacillus: Five strains resembling this organism by cultural and biochemical characteristics were isolated from four sick dogs, whereas no strain so typical of these was found in normal dogs. These strains were considered to be associated with complications of distemper.

7. Non-haemolytic streptococci: These organisms were recovered from 73.3 per cent. of the normal and 96 per cent. of the sick dogs. Representatives of *Str. salivarius*, *Str. equinus*, *Str. bovis* and *Str. faecalis*, were amongst the 133 strains isolated in this investigation.

8. Coliform bacilli: The frequency of this group of organisms was considerably higher in sick (48 per cent.) than in normal dogs (21.3 per cent.). Of the thirty-two strains, fourteen were typical *B. coli*, twelve were aerogenes or atypical forms and six were of *B. cloacae* type.

9. Bacterium alkaligenes: Fifty-eight strains belonging to this group were isolated from the normal and sick dogs, and their cultural and biochemical characteristics were found similar to those described by Petruschky and Nyberg. The frequency of these organisms in sick dogs (60 per cent.) was markedly higher than that in normal dogs (36 per cent.).

10. Proteus sp./

10. Proteus sp.: Strains belonging to this species were isolated from 16 per cent. of the normal and 28 per cent. of the sick dogs; their frequency in the nose, nasopharynx and tonsils of normal and sick dogs was compared and their probable role as secondary invaders in catarrhal conditions in dogs described.
11. Gram-negative cocci: These were isolated from 60 per cent. of the normal and 56 per cent. of the sick dogs. 67.4 per cent. of the strains belonged to the typical *Neisseria catarrhalis* species; 22.1 per cent. resembled *N. catarrhalis* biochemically, but culturally showed great dissimilarity to "catarrhalis" and so were considered to be a new species similar to that described by Huntoon as "*Neisseria pseudo-catarrhalis*". The remaining 10.5 per cent. belonged to the *N. pharyngis* group. No strain showing pigmentation on culture media was isolated.
12. Diphtheroid bacilli: Either *C. hofmanni* or *C. xerosis* was recovered from 18.7 per cent. of the normal and 28 per cent. of the sick dogs. No pathogenic importance was attached to them.
13. Bacillus subtilis group: These were isolated from 10 per cent. of the normal and 20 per cent. of the sick dogs, mostly from the nose and only in one instance from the tonsils.
14. Haemophilus canis: Only 12 per cent. of the normal and 8 per cent. of the sick dogs showed haemophilic organisms which were identified as *H. canis*. No pathogenic significance was attached to them either.

15. Leptotrichia sp.: Filamentous organisms of the kind isolated from the human mouth were present in the tonsils and nasopharynx of dogs, but no pathogenic significance was attached to them in view of the fact that they were non-pathogenic for laboratory animals, and also by the failure to find significant difference between their occurrence in normal and sick dogs.

16. Unidentified: Three groups of unidentified Gram-negative bacilli were described and their possible generic status discussed.

17. Typical Pasteurella strains were not isolated, neither was the bacillus reported to have been isolated by Perez (Perez's bacillus) from the saliva and nasal mucosa of normal and sick dogs. Similarly, other human pathogens, namely; Str. pneumoniae, C. diphtheriae, H. influenzae and N. meningitidis, which are normal inhabitants of the human nasopharynx, were not found in the nasopharynx of dogs.

HISTORICAL SURVEY.

De Jong (1912) recorded the death of a few of his laboratory cats by the infection of haemolytic streptococci which he recovered from the exudates of the respiratory tracts, but did not describe the characteristics of those streptococci. Again in 1922, Bayne-Jones described an epizootic in cats due to haemolytic streptococci. The disease broke out among twenty-five cats kept in a room in his laboratory and was so fatal that all the cats, with the exception of two, died within a fortnight. The disease began with an infection of the upper respiratory tract, with sneezing and greenish discharge from the nose, followed shortly by a fatal septicaemia. Haemolytic streptococci were recovered from the discharges and blood of the affected cats, most often in pure culture, and he thought that the cause of the malady was haemolytic streptococci alone (not associated with any other organism). He was particularly careful to look for the organisms of the haemorrhagic septicaemia group in these dead animals, but they were not found. All the strains of streptococci isolated from these animals were identical in their cultural and biochemical characteristics. They caused clear beta type of haemolysis on blood agar, failed to ferment mannitol and inulin, produced a final pH of 4.8 in glucose broth and were unable to hydrolyse sodium hippurate/

hippurate. The same author studied the throats of fifty apparently normal cats with a view to determining the frequency of occurrence of haemolytic streptococci in these animals, but was able to isolate it only from one of them, concluding therefrom that it rarely occurred in the nasopharynx of healthy cats (2 per cent.)

Rivers and Bayne-Jones (1923) recorded the isolation of influenza-like bacilli from the throats of seven of the fifteen cats studied. All those strains were Gram-negative, non-motile, non-sporing, small pleomorphic bacilli, morphologically resembling *H. influenzae*. Culturally the organisms would not grow on ordinary media. But unlike *H. influenzae*, all these strains (excepting one) were able to grow on a medium to which an autoclave labile substance (V factor) was added as accessory growth factor. The one exception noted did not grow in any medium which did not contain an autoclaved blood or blood extract. Even the medium containing haematin and yeast extract (X and V factors), which supports a good growth of true influenza bacilli, was of no value in growing this strain. All of them reduced nitrates, some only produced indole and their ability to ferment carbohydrates was variable. Since these strains resembled the strains isolated from the human throat and lung cultures (Rivers, 1922) in their morphology, growth/

growth requirements and biochemical reactions, these also, like the human strains, were assigned to the *Haemophilus parainfluenzae* group.

Kremsreiter (1937) isolated from the throat of a cat a small, non-motile, non-sporing, Gram-negative bacillus which he described as belonging to the haemoglobinophilus group. The biochemical reactions of this organism were similar to *Pasteurella septica*, and it was also pathogenic to experimental animals. An organism which differed nowise from this bacillus was found in the discharge from a wound in a man caused by the bite of that particular cat, indicating the transmission of this organism into the wound from the mouth of the cat.

Similarly, Rimpau, in 1937, reported three instances where haemoglobinophilic organisms similar to the one described by Kremsreiter were recovered from the throats of cats and also from the wounds inflicted by these cats in human beings.

Schenk (1938) isolated *Pasteurella septica* strains from the throats of all of six sick cats and from nine out of fourteen healthy cats which he examined. He also referred to four instances where the same organism was isolated from cat bite wounds in human beings. He believed that his organism was identical with those isolated by Kremsreiter and Rimpau, although these authors had described their organisms/

organisms as being haemoglobinophilic. Schenk described his organism as being a minute- Gram-negative bacillus which showed bipolar staining when isolated from pus or from the blood of an injected rabbit. This characteristic was not evident on agar culture, but pleomorphic forms occurred with degeneration. Colonies were medium-sized, greyish-white and vaulted, having smooth edges and non-haemolytic. Growth was scanty on plain agar and absent on MacConkey agar. No change occurred in litmus milk, but indole was produced and acid without gas was formed in dextrose, galactose, levulose, xylose, mannitol and maltose. The strains isolated from wounds produced no acid from lactose, but certain strains isolated from cats produced acid from that sugar. The organism was pathogenic for laboratory animals.

Allin (1942), while describing three cases of cat bite wound infection with *Pasteurella septica* in human beings, recorded the isolation of that organism from the mouth and throat of the cat which had inflicted the wound in one of his cases.

MATERIAL, TECHNIQUE AND PROCEDURE

Source of material, method of collection and the technique and procedure adopted in this investigation were the same as in the case of the dogs. Thirty apparently normal cats of different breeds and variable age, all destroyed by intracardial injections of nembutol, were selected for swabbing. Sex, breed and age of these animals were not recorded. Three swabs were taken from each animal - one from the nose, one from the nasopharynx and the third from the tonsils - and cultures were made, using the same media as in the case of the dogs but with the addition of a further medium composed of five per cent. blood agar plate with one per cent. glucose and 0.1 per cent. cystine, which was used with a view to the isolation of *Brucella tularensis*, if that organism were present in the nasopharynx of cats.

RESULTS.

The organisms isolated in this investigation are listed in Table LXIII and mostly belonged to the same species as those found in dogs, excepting that typical virulent strains of *Pasteurella septica* and para-influenza bacilli were isolated quite frequently. These organisms, found in cats, were not found in dogs, whereas *H. bronchisepticus*, which was recovered from a small percentage of the apparently normal dogs, was not isolated from these animals. The frequencies of different organisms in all the situations were considerably lower than similar organisms in the corresponding parts in dogs, and the growths too, on the primary culture plates were decidedly less copious.

As per the morphology, cultural characteristics and biochemical reactions, all the organisms isolated in this investigation were roughly classified as follows:-

1. Staphylococci.
2. Haemolytic streptococci.
3. Influenza group bacilli.
4. *Pasteurella septica*.
5. Non-haemolytic streptococci.
6. Gram-negative cocci (*Neisseria*).
7. Diphtheroid bacilli.
8. *Bacterium alkaligenes*.
9. Coliform bacilli.
10. *Leptotrichia* sp.
11. Unidentified.

The absolute and relative frequencies of these organisms in the nose, nasopharynx and tonsils of thirty cats are shown in Table LXI, 1, and since most of these organisms have been described in detail in Part I in connection with dogs, they are only briefly described here, excepting parainfluenza bacilli and *Pasteurella septica*, which were not found in dogs. Staphylococci.

These organisms were recovered from fourteen (46.7 per cent.) of the thirty cats, from the nose of every positive case, from the nasopharynx of three and from the tonsils of two. Only three strains (15N₁, 25N₂, and 25NP₁) were positive for coagulase production in rabbit plasma. So, taking coagulase production as the criterion of pathogenicity, only a small percentage (6.7 per cent.) of the normal cats harboured pathogenic strains. The proteolytic power of these strains was very feeble. Only one strain liquefied solid serum and six of them liquefied gelatin. Ten strains (about 50 per cent.) fermented mannitol. Only four strains showed distinct aureus pigment while the rest were all albus.

Haemolytic Streptococci.

Only three (10 per cent.) of the thirty cats were found carriers of these organisms, one in the nasopharynx and two in the tonsils. All the three strains belonged to Lancefield's serological group G and none of them cross-precipitated with group C serum. The biochemical/

TABLE LXI

Characteristics of Staphylococci (Cats)

S strain	Pigmentation	Biochemical		Coagulase (Rabbit plasma)	Liquefaction of gelatin	Liquefaction of solid serum
		Lactose	Mannitol			
1 N1	Albus	A	A	-	-	-
3 N1	Albus	A	A	-	-	-
3 T1	Albus	A	-	-	-	-
5 N1	Albus	A	-	-	-	-
6 N1	Albus	A	A	-	+	-
7 N1	Albus	A	A	-	+	-
7 NP3	Albus	A	A	-	+	-
9 N1	Aureus	A	A	-	-	-
10 N1	Albus	A	-	-	-	-
14 N1	Albus	A	-	-	-	+
15 N1	Albus	A	-	+	+	-
16 N1	Albus	A	A	-	+	-
16 T2	Albus	A	A	-	+	-
19 N1	Aureus	A	-	-	-	-
19 NP4	Albus	A	-	-	-	-
24 N2	Albus	-	-	-	-	-
25 N2	Aureus	A	A	+++	-	-
25 NP1	Aureus	A	A	+++	-	-
28 N1	Albus	A	-	-	-	-

A (under Sugar) = Acid production.
 - " " = No reaction.
 +++ (under Coagulase) = Positive reaction 3 hours.
 + " " = Positive reaction after leaving over-
 night.

TABLE LXII

Biochemical reactions of haemolytic Streptococci, group G

Strain	Sodium Hippurate	Aesculin	Litmus Milk	Lactose	Raffinose	Glycerol
1 NP1	-	-	AC	A	-	-
11 T1	-	-	A	A	-	-
18 T3	-	-	A	A	-	-

A = Acid product.

- = No reaction

AC = Acid and clot

biochemical reactions of these strains differed in one respect from the strains from dogs and human sources, namely, in the absence of reaction in aesculin, which is in contrast to dogs' and human strains, which have always been recorded as having action on that sugar. None of the strains hydrolysed sodium hippurate and they had no action on raffinose and glycerol. One strain clotted litmus milk with the formation of acid, and the other two formed only acid in that medium. So, as per the biochemical reactions it was not possible to classify them under any of the three types described by Smith and Sherman (1938).

Influenza group bacilli.

All strains of small Gram-negative bacilli which were confirmed as haemophilic by their inability to grow on any of the blood-free media, such as glucose broth, serum broth, serum agar, liver infusion agar, Dorset's egg medium, etc., were studied for the requirements of their accessory growth factors by testing their ability to grow in the presence of haematin, representing the X factor, and unautoclaved yeast extract, representing the V factor, and haematin and yeast extract, representing both X and V factors. They were also studied for the formation of indole and the reduction of nitrate by the methods already described in Part I in connection with *Haemophilus canis*, but their action on carbohydrates was not studied.

Organisms belonging to this group were isolated from nine out of thirty cats (30 per cent.). Two were positive in the nose, seven in the nasopharynx and five in the tonsils.

The characteristics of the fourteen strains isolated in this investigation were not uniform. All of them grew in haemopeptone water and on agar with yeast extract and haematin. All but two strains were able to grow in the presence of yeast extract alone, but failed to grow on media containing haematin only, indicating thereby that these strains needed exclusively the V factor and not the X. Two exceptions noted (23T_e and 29 NP₁), which failed to grow in the presence of yeast extract were able to grow on agar with haematin, indicating in this case that X factor only was essential and not V. Further, both these strains formed indole in haemopeptone water culture. Two more strains (2NP₁ and 2T₂) also produced indole. The reduction of nitrate was the common feature of all the strains. Three strains (12NP₃, 26N₁ and 26T₅) showed haemolysis on blood agar.

The type of growth of these strains in general on chocolate agar were somewhat similar to *H. influenzae*, but on careful observation and on longer incubation it was noticed that they grew on this medium a little more profusely, and the colonies of some of the strains were slightly bigger. Most of the strains grew equally well on ordinary blood agar.

TABLE LXIII

Characteristics of the "influenza group bacilli" isolated from normal cats.

Strain	Haemolysis on 10% horse blood agar plate	Formation of indole	Reduction of nitrate	Growth on agar with 2% haematin (X factor only)	Growth on agar with 10% unautoclaved yeast extract (V factor only)	Growth on agar with haematin and unautoclaved yeast extract (X and V factors)	Growth in haemo-peptone water
2 NP1	-	+	+	-	+	+	+
2 T2	-	+	+	-	+	+	+
7 NP4	-	-	+	-	+	+	+
12 NP3	+	-	+	-	+	+	+
14 NP2	-	-	+	-	+	+	+
14 T1	-	-	+	-	+	+	+
17 N1	-	-	+	-	+	+	+
17 NP1	-	-	+	-	+	+	+
20 NP2	-	-	+	-	+	+	+
20 T1	-	-	+	-	+	+	+
23 T6	-	+	+	+	-	+	+
26 N1	+	-	+	-	+	+	+
26 T5	+	-	+	-	+	+	+
29 NP1	-	+	+	+	-	+	+

The colonies on blood agar were discrete, small, convex or slightly flat, round with entire edge, transparent or slightly opaque, smooth and emulsifiable.

These strains had a tendency to die out in culture more quickly than *H. influenzae*. Some lost their viability even within four days on blood agar at room temperature.

Pasteurella septica.

Organisms identified as *Pasteurella septica* by their morphology, cultural characteristics, biochemical reactions and pathogenicity tests, were isolated from thirteen (43.3 per cent.) of the thirty apparently healthy cats. Eight of them were positive in the nasopharynx, nine in the tonsils and none in the nose. In four animals they were isolated both from the nasopharynx and tonsils.

Morphologically, these organisms were small, coccoid or slightly ovoid bacilli, 0.5 to 1.2 by 0.2 to 0.5 micron, with slightly convex sides and rounded ends. Some organisms looked almost like cocci and some showed slight pleomorphism, appearing like short rods. They were non-motile, non-capsulated, non-sporing, Gram-negative and non-acid-fast; arranged singly, in pairs or in groups but never in chains. Films from cultures stained by Gram's method did not show typical bipolar organisms, even though they appeared deeply stained at the ends, but by Leishman's or Giemsa's stains a few distinct bipolar organisms were seen.

The cultural characteristics of all these strains were quite uniform and no appreciable difference was noticed among them. On blood agar plates, in 24 hours at 37°C. they showed round, low, convex, amorphous, greyish-white, translucent or slightly opaque small colonies, 0.5 to 1.5 mm. in diameter with smooth glistening surface and entire edge; butyrous in consistency and easily emulsifiable without haemolysis.

Growth on nutrient agar was poor. Very small, round, discrete colonies were seen in twenty-four hours.

In broth, moderate uniform turbidity with granular growth was noticed in twenty-four hours. There was no deposit and no surface ring or pellicle, but on longer incubation a slight viscous deposit was seen.

Moderate whitish, fairly confluent growth was noticed in Löffler's serum - no liquefaction. None of these strains showed any visible growth on potato or on MacConkey. Similarly, no growth was observed in gelatin stab culture.

The biochemical characteristics of all these strains were not quite uniform; minor differences were found as shown in Table LXIV. Only eight strains (47 per cent.) were weakly positive for catalase and the rest were negative. None of them formed ammonia or hydrogen sulphide. All produced indole and were negative for M.R. and V.P. Litmus milk was practically unchanged in all cases and methylene blue was not reduced. All without exception produced /

TABLE LXIV.

Biochemical reactions of *Pasteurella septica* (cat)

Strain	Catalase	NH ₃	H ₂ S	Nitrate	Indole	M.R.	V.P.	Methylene blue	Litmus milk	Glucose	Lactose	Mannitol	Inositol	Maltose	Sucrose	Dulcitol	Salicin	Arabinose	Raffinose	Trehalose	Inulin	Dextrin	Rhamnose	Xylose	Sorbitol		
1 NP5	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	A	
2 T1	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
4 NP4	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
8 NP6	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
8 T3	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
11 NP2	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
11 T2	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
13 T3	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
17 NP3	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
17 T2	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
20 NP5	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
22 T3	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
24 NP3	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
26 T4	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
27 NP4	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
27 T3	+	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A
29 T5	-	-	-	+	+	-	-	-	-	V	-	V	-	A	A	-	-	-	-	A	-	-	-	-	-	-	A

produced acid in glucose, mannitol and sucrose, but none of them had any action on lactose, inositol, dulcitol, salicin or inulin. Reactions on other sugars were variable; Xylose and trehalose were acted upon by most of the strains; three exceptions were recorded in the case of the former and four in the latter. Six acted on sorbitol, three on maltose, four on arabinose and two on dextrin.

Freshly isolated strains were tested for pathogenicity to mice, guinea pigs and rabbits. 0.25-0.5 c.c. of the 48 hours' broth culture injected intraperitoneally killed the mice in 24-48 hours, and the organisms were recovered in cultures from liver, spleen and heart blood and were also found in direct smears.

Similarly, 1.0 c.c. of each culture inoculated intraperitoneally to guinea pigs and the same dose intravenously to rabbits was lethal to those animals within 18-36 hours, the organisms being subsequently recovered from the tissues.

Non-haemolytic streptococci.

Of the thirty cats, ten were found positive for these organisms (33.3 per cent.); two of them harboured them in the nose, five in the nasopharynx and six in the tonsils. Out of the thirteen strains isolated, five did not show any haemolysis on horse blood agar plate, and the remaining eight showed alpha haemolysis. The biochemical reactions of these/

TABLE LXV

Non-haemolytic Streptococci (Cat)
Biochemical Reactions

Strain	Haemolysis	Ammonia in peptone water	Growth on MacConkey's bile salt agar	Litmus milk	Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Probable species
2 NP1	Nil	-	-	A	-	A	A	A	-	-	-	-	-	-	-	Salivar- ius
3 T2	Alpha	-	-	AC	-	A	A	A	-	A	-	-	-	-	A	"
4 NP3	Alpha	-	-	A	-	A	A	A	-	A	-	-	-	-	A	"
4 T2	Nil	-	-	A	-	A	A	A	-	A	-	-	-	-	A	"
9 N2	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	A	equinus
12 NP3	Alpha	-	-	A	-	A	A	A	-	A	-	A	-	-	-	Sali- varius
18 T3	Alpha	-	-	A	-	A	A	-	-	A	-	-	-	-	-	Equinus
21 T2	Nil	-	-	AC	-	A	A	A	-	A	-	A	-	-	A	Sali- varius
23 NP2	Nil	-	-	A	-	A	A	A	-	-	-	-	-	-	A	"
23 T1	Nil	-	-	AC	-	A	A	A	-	-	-	-	-	-	A	"
27 NP4	Alpha	-	-	A	-	A	A	-	-	-	-	-	-	-	-	equinus
30 N3	Alpha	-	-	AC	-	A	A	A	-	A	-	-	-	-	-	Sali- varius
30 T4	Alpha	-	-	AC	-	A	A	A	-	A	-	-	-	-	-	"

A = Acid

AC = Acid and clot

these strains were not uniform, but they were somewhat similar to the strains isolated from dogs. Ten strains were roughly classified as *Str. salivarius* and the remaining three as *Str. equinus*. No strain corresponding to either *Str. bovis* or *Str. faecalis* was met with.

Gram-negative cocci.

Only six (23.3 per cent.) of the thirty cats were found positive for these organisms, in two instances in the nose, three in the nasopharynx and three in the tonsils. Out of the eight strains, seven were typical of *Neisseria catarrhalis*, growing profusely on ordinary media and having no action on any of the carbohydrates, simulating *catarrhalis* I (isolated from dogs), while the remaining one, though resembling typical *catarrhalis* in its biochemical reactions, culturally was similar to *catarrhalis* II found in dogs.

Diphtheroid bacillus.

An organism resembling *Corynebacterium hofmanni* was isolated from three (10 per cent.) of the thirty cats, once from the nose and twice from the nasopharynx. These strains grew profusely on ordinary media, showed no metachromatic granules and had no action on any of the carbohydrates.

Bacterium alkaligenes.

These were isolated from five (16.7 per cent.) of the cats. One animal was positive in the nose, three in the nasopharynx and two in the tonsils. All the strains were similar to the type described by Nyberg (1935). They were all non-motile and had no action on any of the carbohydrates or on litmus milk. No strain typical of *Bacterium faecalis alkaligenes* of Petruschky's description (motile and rendering litmus milk strongly alkaline) was met with.

Coliform bacilli.

Three of the thirty cats (10 per cent.) were found positive for these organisms; two strains, one isolated from the tonsils of cat No. 4 and the other from the nasopharynx of No. 20 were the atypical strains of *B. coli* which were identified as *B. lactis aerogenes* on account of their action on inositol, absence of indole production, positive V.P. and negative M.R. reaction, and the presence of growth in citrate medium; while cat No. 21 harboured typical *B. coli* strains in all the three situations (nose, nasopharynx and tonsils). These strains did not ferment inositol, produced indole, showed positive M.R. and negative V.P. reaction and failed to utilise citrate.

Leptotrichia (sp.)

Organisms belonging to this group were as common in these animals as they were in dogs. They were isolated from eight out of thirty cats (26.7 per cent.). In five of them they were present in the nasopharynx and in six in the tonsils, but in none in the nose. All the eleven strains isolated in this investigation were identical with the strains isolated from dogs (described in Part I) regarding morphology, cultural and biochemical characteristics.

Unidentified group (a).

A Gram-negative bacillus of similar cultural and biochemical characteristics to the organism isolated from dogs and described under Unidentified group (a) in Part I, was isolated from 46.7 per cent. of the cats, mostly from the tonsils and nasopharynx and occasionally from the nose.

Unidentified group (b).

A Gram-negative cocco-bacillus of the type described under Unidentified group (b) in Part I, provisionally grouped under the genus bacterium was isolated from only 10 per cent. of the cats.

A few other strains of unidentified Gram-negative bacilli which were occasionally met with and considered to be saprophytes were not recorded.

DISCUSSION.

Staphylococci . No reference is available of any previous study of the staphylococci from cats, and it is now clear from this investigation that, in contrast to dogs, cats very occasionally harbour pathogenic strains, although the presence of non-coagulase strains is fairly common. Aureus pigmentation is no indication of pathogenicity, as the two strains (9N₁ and 19N₁) which showed clear golden pigment failed to clot rabbit plasma and one albus strain (15N₁) was positive for coagulase. Similarly, mannitol fermentation was not correlated with pathogenicity as several coagulase-negative strains also fermented this sugar.

Haemolytic streptococci. This investigation supports the observations recorded by Bayne-Jones (1922) that haemolytic streptococci are rare in healthy cats. Their frequency in respiratory diseases needs to be studied in order to confirm whether or not they are capable of causing septicaemic diseases in these animals as recorded by De Jong (1912) and Bayne-Jones. No instance of the kind appears to have been recorded in recent years, and it may be useful to study the association of these organisms in respiratory and other diseases of cats, as has been done with dogs by Hare.

Influenza group bacilli In 1903 Friedberger recovered from the preputial secretions of dogs an organism which resembled *B. influenzae* in its morphology and in its dependence on blood for culture. It was called *B. haemoglobinophilus canis*. Rivers (1922) tested this organism for its growth requirements and recorded that it demanded only the X factor. In the same year he isolated from two cases of influenza in human beings an organism which was in every respect identical with *B. influenzae*, except that it required only the V factor for growth. He named it "*Bacillus parainfluenzae*". In addition, Rivers and Bayne-Jones (1923) isolated from the throats of healthy cats a haemophilic organism similar to *B. parainfluenzae* regarding its morphology and growth requirements. Fildes (1923) examined the growth requirements of *B. influenzae*, the Koch-Weeks bacillus, *Bacillus haemoglobinophilus canis* and other bacilli and found that the first two were identical, both requiring X and V factors, while the third required only the X factor. He considered that only these three organisms should be included in the haemoglobinophilic group and others described as haemophilic, e.g., *B. pertussis*, should be excluded.

Bacillus influenzae, from the time of its discovery, was generally recognised as non-haemolytic. But Pritchett and Stillman (1919) noted the occurrence among a large number of cultures of haemophilic bacilli isolated from cases of influenza and from

normal persons, of a small proportion of strains which produced haemolysis on blood agar. Rivers and Lanschner (1921) regarded it as a haemolytic variety of the influenza bacillus and as belonging to that group. But it was Fildes (1924) who proved that these haemolytic strains demand only the V factor for growth, being indifferent to the X factor. He worked with fourteen Danish strains of haemolytic influenza bacilli and concluded that they were indifferent to the blood pigment factor but demanded the V factor. In this respect they were similar to the *Bacillus parainfluenzae* isolated from human and cats' throats, which, however, was non-haemolytic. Fildes included the following under what he called, "Influenza group bacilli":-

1. *B. influenzae*, requiring both the X and V factors.
2. *B. haemoglobinophilus canis*, requiring only the X factor.
3. Haemolytic influenza bacillus, requiring only the V factor.
4. *B. parainfluenzae* (Rivers), also requiring only the V factor.

Valentine and Rivers (1927) reported that the majority of the haemolytic strains of haemophilic bacilli/

bacilli found in the human nasopharynx required only the V factor for their growth, while a minority required both the X and V factors, and a proportion of non-haemolytic strains of haemophilic bacilli also required only the V factor, and so they proposed the name, *H. parainfluenzae* for all the haemophilic organisms requiring only the V factor, whether haemolytic or not.

The results of this investigation not only confirm the findings of Rivers and Bayne-Jones of the presence of non-haemolytic strains of *H. parainfluenzae* in the respiratory tracts of cats, but it also furnishes additional information that both the haemolytic strains of the influenza bacillus (12NP₃, 28N₁, and 26T₅) requiring only the V factor for growth (included in the parainfluenzae group, Valentine and Rivers, loc. Cit.) and the strains resembling *H. canis* (23T₆ and 29NP₁) needing only the X factor, are also occasionally found in these animals. . . In other words, it may be emphasised that with the exception of Pfeiffer's bacillus, which requires both the X and V factors, the remaining types included under influenza group bacilli by Fildes are recoverable from cats.

H. parainfluenzae is said to be occasionally associated with pharyngitis, it is a rare cause of ulcerative endocarditis and is occasionally found in infected wounds and sinuses in human beings (Topley and Wilson, 1948b). Since this organism is fairly common/

common in human beings and cats, cross infection between these pet animals and the members of the household appears to be possible.

It is not known what part this organism plays in the respiratory or other diseases of cats, but since mild pathogenicity is attributed to it in human beings, it may similarly cause such conditions in cats and also be associated with some of the respiratory diseases in these animals, either independently or in conjunction with other organisms.

This study further confirms the views of Rivers and Bayne-Jones that any one engaged in studying respiratory epizootics of animals should study the nutritive requirements of any small Gram-negative bacillus which one might come across, lest it should be mistaken for *H. influenzae* or *H. pertussis*, and the fact that such bacilli are present in animals should be recognised by research workers who attempt to reproduce diseases in animals with *H. influenzae* or *H. pertussis*.

Pasteurella septica. Although it appears from the literature that no systematic study has yet been made to investigate the presence of *Pasteurella* organisms in cats with a view to ascertaining their aetiological significance in any of the respiratory or other diseases of cats, yet by way of investigating the possible source of *Pasteurella* infection in wounds/

wounds following cat bites in humans, some authors have studied the throats of cats and isolated *Pasteurella septica* strains from these animals (Kremsreiter, 1937; Rimpau, 1937; Schenk, 1933, and Allin, 1942). Even though Kremsreiter and Rimpau described their organisms as haemoglobinophilic, they were not definite that their strains were absolute in their haemoglobin requirements and, since they were in other respects identical with the organisms isolated by Schenk and Allin and those isolated in this investigation, it may be presumed that Kremsreiter and Rimpau were also dealing with the same organism. These authors probably thought that the strains were haemoglobinophilic on account of the poor growth on agar as reported by Schenk and Allin and also evidenced in this study.

Even though this organism appears to be a harmless parasite in the throats of cats, it is definitely pathogenic when inoculated into guinea pigs, mice and rabbits (other animals not tried). Reports are also available of its pathogenicity to human subjects. Kapel and Holm (1930) first recorded a case of wound infection with *Pasteurella septica* in man following a cat bite. Their observation has since then been confirmed by other workers on the Continent, Kremsreiter, Rimpau and Schenk; in America by Allin (1942) and Hansman and Tully (1945) and also in Britain/

Britain by Allott et al (1944). All these authors agree that the *Pasteurella septica* organism is transmitted from the cat to human beings by its bite or scratch, and such infected wounds give rise to symptoms of pain, swelling and abscess formation, often with complications of subacute osteomyelitis and cellulitis with adenitis. In one of the cases reported by Allin a subperiosteal abscess developed, due to the same organism at the site of a cat bite eleven months after the wound had been inflicted, indicating that the organism remains dormant in the tissues, particularly bone, for months. These authors suggest that this condition should be recognised as a clinical entity and be added to the list of diseases communicable from animals to human beings.

Reports are also available of the isolation of *Pasteurella septica* from various conditions in human beings, such as prolonged puerperal pyrexia (Brugnatelli, 1913), gastroenteritis following the handling of fowls suffering from *Pasteurella* infection (Von Boer, 1916), empyema (Debre, 1919), chronic pneumonia (Foerster, 1938) and appendicular abscess (Ludlum, 1944). But these references are not conclusive enough to confirm that this organism is pathogenic to human beings by other routes than by wounds, such as ingestion and inhalation. However, if/

if by any chance the latter is possible, household cats could easily be the sources of such infections, being apparently frequent throat carriers of this organism.

It is not known whether this organism remains only as a harmless parasite in the throats of cats or if it is capable of causing any pathogenic symptoms in these animals under favourable conditions. An extensive study investigating its association with various respiratory affections would be necessary to decide this factor.

Other organisms. Regarding other organisms, namely, non-haemolytic streptococci, Gram-negative cocci, diphtheroid bacilli, coliform bacilli, leptotrichia sp. and unidentified Gram-negative bacilli, these have been fully discussed in Part I with reference to dogs and, since they do not appear to have any more importance in cats, they are not discussed here again to avoid repetition.

SUMMARY

1. Thirty apparently normal cats, destroyed by intracardial injections of nembutol, were studied for the presence of various micro-organisms in the nose, nasopharynx and tonsils.

2. Staphylococci were isolated from the noses of fourteen (46.7 per cent.), nasopharynx of three (10 per cent.) and from the tonsils of two (6.7 per cent.) cats. Only two animals were found to be carriers of pathogenic (coagulase-positive) strains, one in the nose and the other in the nose and nasopharynx.

3. Three cats (10 per cent.) were positive for haemolytic streptococci, two in the tonsils and one in the nasopharynx. All the three strains belonged to Lancefield's serological group G. None of these strains fermented aesculin, in contrast to group G strains of dogs and human origin, which always act on that sugar.

4. Influenza group bacilli were isolated from nine cats (30 per cent.). Of the fourteen strains, twelve were *H. parainfluenzae* type, requiring only V factor for growth, and the other two needed only X factor and were positive for indole, resembling *H. canis*.

5. *Pasteurella septica* strains were isolated from thirteen cats (43.3 per cent.), either from the nasopharynx or tonsils, or from both, but never from the nose.

6. Non-haemolytic streptococci, Gram-negative cocci, *Corynebacterium hofmanni*, *Bacterium alkali-genes*, Coliform bacilli and *Leptotrichia* sp. were also recovered and their frequencies recorded.

7. Two groups of unidentified Gram-negative bacilli were recorded.

8. *Brucella tularensis* was not isolated.

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