STUDIES ON THE INCIDENCE, DIAGNOSIS AND CONTROL OF BOVINE MASTITIS IN BRITISH COLUMBIA.

- by -

Florence Rosena Tamboline

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of
MASTER OF SCIENCE IN AGRICULTURE
in the
DEPARTMENT OF DAIRYING

Accepted Oct. 19, 1946

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1946.

TABLE OF CONTENTS.

STUDIES ON THE INCIDENCE, DIAGNOSIS AND CONTROL OF BOVINE MASTITIS IN BRITISH COLUMBIA.

INTRODUCTION.

Bovine mastitis is probably responsible for a greater economic loss to the dairy farmer each year than any other single factor. The extent of this loss is variously reported for different parts of the world, depending largely on the scope of the surveys made and on the methods used for the detection of the disease.

The Bureau of Dairy Industry, United States Department of Agriculture, (20) estimated that the dairymen of that country in 1942 lost at least 3% of their potential milk production as a result of mastitis. This would amount to a loss of 3 1/2 billion pounds of milk, which at $1.50 per 100 pounds would be worth $52,500,000.00. Hopson is quoted (1) as having stated in a speech to dairy farmers at Guelph, Ontario, in October, 1945, that in the United States and Canada, cows valued at over $200,000,000.00 are sold every year because they are suffering from mastitis.

Holford (48) in 1930 reported on a study of 5,000 herds in New York State, and on the basis of his findings calculated that mastitis was responsible for an annual loss in the United States of $72,011,455.00. This estimate is

very conservative, since it assumes that only 4.3% of all milk cows are sold each year because of mastitis, that had these animals remained in the herds they would have produced only 68% as much milk as if they had been healthy, and that the average loss in value of the cows discarded is $25.00 per head. Losses due to the reduced milk production of infected cows retained in the herds are not considered. If Holford's results are applied to Canada, on the basis of a milk cow population of 3,930,000 and a total milk production of 17,604,823,000 pounds in 1944, and if $1.82 is taken as the farm value of 100 pounds of milk (22), there is an annual loss attributable to the removal from herds of mastitic animals of $13,613,509.

In order to evaluate fully the financial loss due to mastitis, several factors must be taken into consideration:

(1) the decrease in the amount of milk produced by the infected cow,

(2) the relatively higher cost of feed per pound of milk produced by the infected cow as compared to that for a normal cow,

(3) the decrease in the value of the infected cow, herself, with probable eventual total loss,

(4) the loss due to the production of milk unfit for human consumption and of little or no commercial value.

From the above reports it can readily be seen that

the dairy industry annually sustains a tremendous loss as a result of mastitis infection. Despite the seriousness of this apparently widespread disease, very little work has been done in British Columbia either to study the extent of infection in this province or to try to develop control measures.

The first investigation on mastitis in this province was that reported by Berry and Clark (6), who in 1936-37 made a study of 53 cows from several herds in the Lower Fraser Valley. These workers confined their study to specified groups of cows, and in their report presented an evaluation of the relative merits of various field and laboratory tests for the detection of the disease as applied to the selected groups of animals.

Because of the severity of the disease in the dairy herds of British Columbia, with its resulting reduction in the revenue of the province, the British Columbia Industrial and Scientific Research Council, through its Technical Advisory Committee on Agriculture, established a Mastitis Sub-Committee to study the clinical and laboratory detection of the disease, as well as to devise methods for its control and eradication. This Sub-Committee, under the chairmanship of Dr. J.G. Jervis, is composed of representatives of the Dominion and Provincial Departments of Agriculture, the British Columbia Veterinary Association, milk distributing organiza-

tions, dairy farmers, and the University of British Columbia Departments of Dairying and Animal Husbandry.

The work of the Research Council on mastitis began in May, 1944. The first undertaking of the Council was a preliminary study of the prevalence of mastitis in the North Okanagan Valley. This survey was carried out in conjunction with work which had been proceeding under a University Research Fund Grant on problems associated with cheese-making in the North Okanagan Valley. A period of two months was devoted to this study.

During the following twelve months, a general survey was made of the incidence of the disease in British Columbia. Milk samples from clinically infected and suspicious cows were taken by co-operating veterinarians and forwarded to the laboratory for examination. The majority of the samples were obtained from the Lower Fraser Valley. However, a number were received from Interior points and from Vancouver Island.

In order to acquaint the dairy farmer with the project, and to encourage him to adopt the best possible sanitary methods to prevent the spread of the disease in his herd, a Handbook (43) was prepared by the Committee, and distributed throughout the province by milk processing plants, dairy associations and practising veterinarians.

Following the completion of the general survey, an intensive study was made of a few herds, in which a rigid

management program had been initiated. This work was undertaken with the object of demonstrating that most forms of mastitis can be held in check and eventually eradicated from a herd by careful management practices. A detailed study of the types of organisms present in the milk from the cows of the selected herds was made in order to determine the nature of the flora in specific herds and in certain areas. The classification of the organisms was also used as a basis for experimental treatment studies. Throughout the work, particular attention has been paid to the evaluation of the various procedures employed for the detection of mastitis, and of the biochemical and serological techniques used for the classification of the organisms associated with the disease.

It is the purpose of this report to review the significant findings of the work undertaken by the Research Council on mastitis together with a summary of the literature pertaining specifically to the program of study of the Council.

HISTORICAL OUTLINE.

In the present report, no attempt has been made to enumerate all of the papers on mastitis. Reference is made only to those articles considered pertinent to the work described herein.

A complete review of the extensive literature on

mastitis up to the end of 1935 may be found in the monograph by Munch-Petersen (75). A more recent survey of the field is that of Merchant and Packer in 1944 (68). Although having fewer references to specific articles, the latter publication gives a condensed summary of all aspects of the problem.

Forms of Mastitis.

Mastitis is generally defined as any inflammation of the udder. The disease may be non-infectious or infectious in nature.

The non-infectious form is due to such factors as congestion at freshening, chilling, bruising and injury of the teats and udder. The condition usually clears up as soon as the causative factor has been corrected, provided that the remedial measures are not too long delayed. This type of mastitis may, however, leave the udder in a weakened condition, predisposing it to infection by opportunist bacteria.

Infectious bovine mastitis is caused by various species of bacteria and may be divided into two distinct types: acute and chronic. Bryan (12) further divides the acute form into acute local and acute systemic mastitis. Acute local mastitis is characterized by swelling of the udder accompanied by pain and the production of milk of abnormal physical appearance. All of the symptoms are confined to the udder. In

acute systemic mastitis, the cow shows general signs of illness in addition to the symptoms of acute local mastitis. The acute systemic type, although relatively rare, is very often fatal. Acute local mastitis may get progressively more serious, becoming acute systemic. Acute systemic mastitis results when the causative organism metastasizes from the udder into the blood stream. In some cases, only the toxic by-products of the organism diffuse into the general circulation.

In some instances, acute mastitis may become gangrenous, resulting in the death of the animal, or, if recovery takes place, in the sloughing off of the affected quarter.

Acute mastitis invariably results in one of the following four conditions:

(1) the animal may die,

(2) the animal may completely recover, either spontaneously or in response to treatment,

(3) the affected quarter or quarters may atrophy and become blind,

(4) chronic mastitis may develop. The organism causing the acute form may, itself, be responsible for the chronic condition, or the acute attack may predispose the udder to infection by the less virulent organisms associated with the chronic type of mastitis.

Chronic mastitis is by far the most common form of

the disease. The condition develops very slowly and may exist in an animal for some time without any visible signs of its presence. The udder and its secretion are normal in physical appearance. As the disease progresses, the secretory cells of the udder are destroyed and are eventually replaced by connective tissue. Areas of fibrosis or induration can now be detected on palpation of the udder. In the advanced stages of the disease, almost all of the secretory tissue is destroyed, rendering the quarter non-functional for milk production. One of the characteristics of the chronic form of mastitis is the occurrence of periodic flare-ups of acute mastitis. These flare-ups may be manifested merely by the secretion of flaky or clotty milk for a few milkings, or the animal may show symptoms of acute local or acute systemic mastitis. Between flare-ups the milk is usually of normal physical appearance.

Because of its insidious nature, chronic mastitis is less readily detected than acute mastitis. Until the disease has progressed to the point of the formation of fibrosis or induration in the udder, or there is an acute flare-up, it is impossible to detect chronic mastitis except by a chemical and bacteriological examination of the milk.

Non-infectious mastitis occurs much less frequently and is responsible for much less serious consequences than the infectious type. In the studies described herein, no known cases of non-infectious mastitis have been encountered. Thus the term "mastitis" has been used in the succeeding

parts of this report to refer to the infectious form of the disease only.

Bacteriology of Mastitis.

The fact that mastitis is not a simple disease but is manifested by several distinct forms, may be attributed, in part at least, to the great variety of organisms which have been shown to be capable of producing an inflammation of the udder. A certain set of symptoms may be consistently found with a particular organism. The action of another organism, however, may result in a variety of symptoms on different occasions depending on the part played by contributory factors. Further, a number of different organisms may all be associated with the same set of symptoms. These facts explain many of the difficulties encountered in the accurate clinical and laboratory diagnosis of the disease, and hence in the prescribing of adequate control and treatment measures.

1. Bacteria causing mastitis.

In general, chronic mastitis is caused by several species of streptococci and less frequently by staphylococci. Acute mastitis is caused by a number of organisms, including staphylococci, some species of coliform bacteria, Corynebacterium pyogenes and Pseudomonas aeruginosa.

Streptococci have been incriminated as the etiological agents of mastitis in the great majority of cases.

Various workers report up to 90% of mastitis infections to be caused by these organisms. There are four species of streptococci which have been definitely proven to be capable of producing mastitis.

Streptococcus agalactiae Lehmann and Neumann, which is also known as Str. mastitidis Migula, has come to be recognized as the most common cause of chronic mastitis. There is no indication in the literature that this organism ever produces isolated acute cases. An acute attack associated with this organism may be a flare-up of an already established chronic condition or it may mark the beginning of a chronic infection.

Str. agalactiae is characterized by a low invasive power and can apparently live in the udder for considerable lengths of time without producing any demonstrable pathological changes. This has been shown in work done in Australia (16) where this organism was isolated for varying periods of time from the milk of animals in their first and second lactation periods. In no case was there any indication of mastitis. Evidence suggests that injury to the udder or some other debilitating factor is necessary before infection by Str. agalactiae can take place. In fact, the ease with which this organism can be isolated from the milk of apparently normal cows, has led some authors (16) to the conclusion that Str. agalactiae should be considered a normal inhabitant of the udder, and that the development of

disease is due to decreased resistance on the part of the host and not to the activity of the organism.

It should be noted at this point, that, because Str. agalactiae had been shown in the early studies on mastitis to be responsible for up to 99% of the cases, diagnostic procedures have been developed for the sole detection of this organism. As a result, in many of the more recent studies, cases of sub-clinical mastitis due to organisms other than Str. agalactiae have been overlooked, because the methods used did not detect these organisms.

There are two other species of streptococci which are quite similar to Str. agalactiae and which frequently cause mastitis - Str. dysgalactiae (Minett Group II) and Str. uberis (Minett Group III). Merchant and Packer (68) found Str. agalactiae to be responsible for about 90% of cases of streptococcic mastitis in Iowa and Str. dysgalactiae and Str. uberis for about 5% each.

Minett et al (74) described the condition produced by Str. dysgalactiae as being severe and more acute than that caused by Str. agalactiae. In some cases speedy destruction of the involved quarter results, while in others recovery takes place. Rarely is an infection of a more or less permanent nature produced. Mastitis caused by Str. dysgalactiae appears to be less contagious than that produced by Str. agalactiae, and is usually limited to isolated cases in a herd.

Str. uberis infection, on the other hand, is generally mild and may be of a transient or somewhat chronic nature (74). Only very slight changes are produced in the milk of the affected quarter by this organism.

Another streptococcus occasionally incriminated in mastitis is Str. pyogenes Rosenbach, the organism responsible for scarlet fever, septic sore throat, erysipelas and related infections in man. Mastitis due to this organism is frequently of the acute type and is thus readily detected. It may, however, be of a sub-clinical nature and evade detection until an outbreak of disease in man, caused by the use of raw milk containing this organism, has been traced to the particular infected animal.

Merchant and Packer (68) appear to consider Str. zooepidemicus ("animal pyogenes") an etiological agent of mastitis worthy of note. These workers state that this organism causes a very severe and often systemic attack which is frequently fatal. They report further that many workers believe that Str. zooepidemicus is responsible for most cases of streptococcic mastitis in which the organism metastasizes from the udder. Little mention of this organism as an important agent in mastitis infection has been found in the reports of other workers. However, in view of the fact that both Str. dysgalactiae and Str. zooepidemicus belong to Lancefield's serological Group C (102) it is possible that

some workers may have reported all Group C infections to be due to the former organism without further differentiation.

Various investigators have reported several other species of streptococci to be associated with occasional cases of mastitis. When the methods of isolation of the organisms and the circumstances relating to the specific cases are considered, it is probable that such attacks represent chance infections. It may be that the streptococci are secondary invaders only, or that factors other than bacterial invasion are of greater importance in the initiation of the disease.

The extent to which staphylococci are responsible for mastitis is variously reported by different investigators. A complicating factor in the diagnosis of staphylococcic mastitis is the fact that an undefined number of species of staphylococci ("udder micrococci") form part of the normal flora of the udder. Even hemolytic staphylococci are frequently found in the udder with no evidence of disease (34). Thus it is very difficult to determine whether a staphylococcus isolated from the milk of a suspected cow is responsible for the condition, or is present merely as a normal inhabitant of the udder. Further, it is difficult to determine whether or not the presence of considerable numbers of staphylococci in apparently normal udders represents the initial stages of sub-clinical mastitis. It is probable that the actual initiation of disease, as in the case of mastitis

caused by Str. agalactiae, is brought about by other factors and that the staphylococci are merely opportunist in activity.

Staphylococci have been most frequently incriminated as the causative agents of an acute mastitis, especially in recently freshened cows. The disease is often fatal (70). Many workers, however, are of the opinion that more cases of chronic mastitis are due to staphylococci than have heretofore been considered. Chronic mastitis caused by staphylococci is usually very mild , and is often less severe than that produced by Str. agalactiae.

Certain members of the coliform group have been found to be etiological agents of mastitis. Murphy and Hanson (78) in a three-year study of 120 cows encountered 79 cases of mastitis due to coliform organisms. Aerobacter aerogenes was isolated from 59% of the cases, "intermediate" types from 26% and Escherichia coli from 15%. The coliform organisms usually cause a very acute attack of short duration. There is generally complete recovery, although Ferguson (35) found a number of cases of mastitis due to coliform organisms to terminate fatally. On some occasions the attack is of a milder and more chronic mature. Coliform mastitis is usually sporadic and does not spread extensively throughout a herd. Burkhardt et al (21), however, reported an outbreak of severe acute systemic mastitis due to A. aerogenes in 11 cows in a herd of 33.

Corynebacteria (diphtheroids) are frequently found in

the flora of the normal udder (34). The only species which has been incriminated in mastitis is Corynebacterium pyogenes. This organism is often encountered in Great Britain and Continental Europe as the cause of the so-called "summer mastitis" which is most prevalent in dry cows and maiden heifers on pasture (71). In North America, C. pyogenes has also been found to cause mastitis, but the condition does not appear to be associated with any particular season of the year nor with any particular stage of the lactation period.

C. pyogenes produces a severe acute or chronic condition accompanied by suppuration and abscessation. The affected quarter frequently sloughs off. Bean et al (4) made a study of 23 quarters of 15 cows infected with C. pyogenes. 22 of these quarters were apparently permanently damaged by the infection, either becoming blind or secreting pus.

Pseudomonas aeruginosa has been found to cause sporadic outbreaks of acute purulent mastitis. Permanent injury of the affected quarter usually results. Cone (26) described an outbreak of mastitis in one herd due to this organism. He found that in some cases the acute attack was followed by a mild chronic condition.

In Europe, mastitis due to Mycobacterium tuberculosis is fairly common, and plays an important role in the spread of tuberculosis in man in areas where raw milk is consumed. On this continent, however, bovine tuberculosis has been

largely eradicated, and so this type of mastitis is not often encountered.

The relationship between Brucella abortus infection and mastitis is not clearly understood. This organism does not appear to produce well defined lesions in the udder. In cases of mastitis where Br. abortus is shed in the milk, other organisms are also usually present. Thus it is difficult to determine whether Br. abortus is the cause of the mastitic condition or whether it has merely predisposed the udder to infection by other disease-producing bacteria.

Most workers attribute gangrenous mastitis to mixed infections of streptococci, staphylococci and coliform organisms. Schalm, however, has reported a number of cases of gangrenous mastitis which he found to be caused by Staphylococcus aureus (96).

Numerous other species of bacteria have been reported in isolated cases of the disease. In most instances it has not been shown conclusively that the incriminated organism was actually the causative factor, and it is likely that in many cases the organism found was merely a secondary invader. On other occasions such attacks are probably chance infections due to decreased resistance on the part of the host, and possibly to increased virulence on the part of the organism.

Hastings and Beach (46) and Johns and Hastings (55) and (56) have reported on studies of cows which were not shedding Sc. agalactiae or other recognized pathogen, but

which repeatedly showed an abnormal pH, percentage chlorine and catalase content in their milk. These workers called this condition "non-specific" mastitis, and found it to be of a mild, progressive, chronic nature, increasing in incidence from lactation to lactation. Peterson and Hastings (81) concluded from their studies that this mild "non-specific" mastitis is the primary infection in chronic mastitis and that the more severe infection due to Str. agalactiae follows after the udder defence mechanisms have been weakened by the primary infection.

As a result of cytological studies, Peterson et al (82) concluded that "non-specific" mastitis is spread from cell to cell along the milk ducts and eventually reaches the udder parenchyma. It is never spread through the milk. These findings may explain why some investigators have observed fibrosis in the udder without any indication of Str. agalactiae infection. Although they were unable to demonstrate inclusion bodies in the udder cells, these authors postulated that "non-specific" mastitis might be caused by a virus.

Broadhurst et al (8) have reported the isolation of a virus from mastitic milk. They report further the finding of inclusion bodies in the leucocytes of the mastitic milk. They claim to have propagated the virus in tissue cultures, to have observed inclusion bodies in the tissues of mice injected with the virus, and to have reisolated the virus

from the mouse tissues. They do not report, however, having tried to infect healthy udders with their virus preparation.

Hoerlein (47) expresses the opinion that the virus theory of mastitis infection has been brought forth in an attempt to eliminate some of the confusion existing in the determination of the etiological agents of this disease. The fact that no organisms can be isolated from many obvious cases of mastitis has, in his opinion, done much to strengthen the virus theory. Because of the lack of definite evidence of the relation of viruses to mastitis, no conclusions can as yet be drawn regarding viruses as possible etiological agents of mastitis.

2. Source of Infective Agents.

There are a number of sources from which the bacteria causing mastitis may originate.

Evidence points to the fact that Str. agalactiae lives almost exclusively in the udder and its secretion. Attempts to isolate this organism from the throats, nostrils, tonsils and vaginas of cows shedding Str. agalactiae in their milk have proven futile (45) and (39). Of interest in this connection is the report from Australia that this organism has been isolated from bovine faeces (16).

Because Str. dysgalactiae and Str. uberis have been less often incriminated as causative agents of mastitis than Str. agalactiae, the possible sources of these organisms

have not been so extensively studied. It is likely, however, that they, too, are found chiefly in the udder.

Str. pyogenes is a human pathogen and infection of the udder of a cow by this organism can almost invariably be traced to a human carrier who has been attending the animal in question.

Merchant and Packer (68) state that Str. zooepidemicus is found in infections of the genital tract of cows.

The staphylococci are much more widely distributed in nature than the mastitic streptococci. They form part of the normal flora of the udder. They are found on the skin and mucous membranes of animals. They are also found in infections of the genital tract of cows (68). Because of the ubiquitous nature of the staphylococci, mastitis caused by this group of organisms is much more difficult to control than that produced by streptococci (70).

The coliform organisms which occasionally invade the udder are probably of faecal origin. A. aerogenes may, however, also come from feed and bedding.

C. Pyogenes is one of the most common organisms associated with internal suppurative conditions in cattle (39). It is found in the respiratory tract and cervical lymph nodes (68). It is likely that exudates from such sources provide the inoculum for cases of mastitis due to this organism.

The sources of the other bacteria which have been

found capable of causing mastitis are not well defined. Most of these organisms are probably normal inhabitants of the cow's environment.

3. Transmission of Infective Agents.

It has been found that, in most cases, mastitic organisms gain access to the udder through the teat canal. Attempts to produce infection by the intravenous injection of mastitic organisms have failed. Miller (69) injected animals intravenously with cultures of Str. agalactiae containing 111 million and 3 billion organisms per c.c. with no resulting infection in the udder. A report from the United States Department of Agriculture (17) describes the failure to induce udder infection by the intravenous injection of 10 c.c. quantities of 24 hour broth cultures of a mastitic staphylococcus.

In considering the spread of mastitic organisms, it is important to remember that each quarter of the cow's udder is a physiological entity, and that infection of a single quarter is quite possible without the other three becoming involved. The transmission of organisms from one quarter to another in an individual animal follows the same course as the spread of infection from one cow to another.

Since chronic mastitis due to Str. agalactiae is the only form of the disease which is actively contagious, and since this organism is found almost exclusively in the

udder, the spread of Str. agalactiae is brought about largely by carelessness during the milking procedure. The other organisms which cause mastitis do not, as a rule, produce epizootic outbreaks of the disease, and are almost universally present in the environment of the teat. With these organisms, the problem of transmission is not very important because they are always in contact with the teat. Thus any control measures instituted in a herd to prevent the spread of the disease will be of value only for Str. agalactiae infections.

The following practices all contribute to the spread of Str. agalactiae: failure to sterilize the milking machine cups between cows, milkers neglecting to sterilize their hands between cows, the use of unsterile cloths to wipe the udders, milking mastitic cows before healthy ones, milking mastitic milk onto the floor or bedding, careless disposal of manure and bedding, careless use of teat plugs, dilators and ointments.

Many workers believe that feeding calves on mastitic milk and then permitting them to suck one another contributes to the incidence of Str. agalactiae infection in heifers. Similarly, it is possible that the practice of letting calves nurse both healthy and mastitic cows may help to spread the organisms to normal animals.

Flies have come to be suspected as agents in the spread of mastitic organisms. Sanders (89) and (90) has

carried out experiments with Musca domestica, the common house fly, and Hippelates sp., the fruit fly, and has shown that these insects are able to carry the organisms in mastitic milk and can implant them in the teat orifice in such a way that infection frequently results. He suggests that a drop of milk is frequently left at the bottom of the teat after milking, that the flies feed on this, and that they then fly to another teat carrying with them any organisms which may have been in the drop of milk. It appears that the insects can cause a slight injury to the teat orifice, thus assisting the organisms to gain entrance to the udder. Sanders found that flies were most efficient in spreading mastitis among dry cows. This he attributed to the fact that in such animals, the udder secretion is not removed and serves as an admirable medium for the growth of the mastitic organisms. These observations help to explain why "summer mastitis" due to C. pyogenes is thought to be spread by flies, subsequent to the cows sucking one another and probably leaving a drop of milk at the apex of the teat.

In the case of Str. pyogenes, the organism is spread from the infected person to the cow by droplet infection as a result of coughing, or by the handling of the udder and teats with infected hands.

4. Mode of Infection.

In the initiation of any disease, there are three

factors to be considered: virulence of the invading organism, numbers of the invading organism, and resistance of the host. There is probably no other disease in which the inter-relationships of these factors is less understood than in the case of mastitis. Numerous explanations have been offered as to why on some occasions infection readily takes place, and why on other occasions under apparently identical conditions it is impossible to induce infection. None of these theories, however, have been able to account for all cases of mastitis.

The conflicting results obtained in experiments on the role of the teat in preventing bacterial invasion of the udder have led to much confusion. There are many reports of intact teats having been dipped in suspensions or cultures of mastitic organisms with no infection resulting. Little (64) suggests that the teats of heifers act as physical barriers to bacterial invasion, but that in older cows the teat sphincter is more patent and the duct dilated, thus more easily permitting bacterial invasion. He was unable to induce infection in heifers by applying cultures of a hemolytic Str. agalactiae to the intact teat, but was able to do so in some older cows. He believes, further, that because of the negative pressure created in the udder during the act of milking, the organisms at the tip of the teat may be drawn into the teat by suction. Other workers have found that organisms applied to the teat orifice can invade the udder if the teat has first been injured in some way. This explains why mastitis so frequently

follows injuries to the teats and udder. Ferguson (36) made a study of the milk from 317 cases of injured quarters and found 89% to have become infected following injury. However, the fact that Str. agalactiae infection can spread through a herd at a more rapid rate than can be accounted for by injury, suggests that other factors, perhaps the virulence of the organism, may play an important role in such cases.

Little (63) found that when udders were inoculated beyond the teat sphincter with Str. agalactiae, the incidence of infection was related to the numbers of organisms introduced. He suggests that the reason for repeated doses of small numbers of organisms being necessary to produce infection, is that in such cases the udder has to be sensitized to the injected organism, and that following sensitization with the first few doses, infection can take place.

It should be pointed out, however, that the reports of many workers regarding the production of acute mastitis following the inoculation of the udder with massive doses of streptococci, should not be considered evidence that the resulting disease is similar to what would have been produced by the same organisms under natural conditions. These experiments show the response of the udder defensive mechanisms to large doses of foreign protein, and do not represent the slow progressive action of the bacteria on the udder tissue as occurs in chronic mastitis.

Gorini (41) believed that the common mastitic organisms

were normal inhabitants of the udder, and that they lived in the udder in a state of equilibrium between the bacteriostatic effect of the udder and the toxins of the organisms. When this equilibrium was upset in favour of the bacteria, disease resulted.

Numerous workers have reported on the bactericidal effect of fresh cow's milk on various organisms. Jones and Little (60) studied the bactericidal action of the milk from different cows on a non-hemolytic mastitic streptococcus and found that different milks differed in their action. In some milks the inhibitory action lasted for eight hours, whereas in others it lasted only four to six hours. They concluded that the inhibitory substance originated in the udder, and suggested that the bactericidal substance or substances may be responsible for the limited flora of the udder, since staphylococci and coliform organisms, which live in such close proximity to the teats, do not survive to any extent nor produce disease in the normal udder. If Jones and Little's assumptions are correct, it is possible that the initiation of mastitis in the udder may be associated with a reduction in the quantity or an alteration in the nature of this bactericidal substance.

It is the writer's opinion that the problem of mastitis control would be much simplified if the mode of infection were better understood. What is the efficiency of the teat in keeping bacteria from entering the udder? Is it that on

certain occasions more organisms than usual pass beyond the teat sphincter and so produce disease, or is it that the udder defensive power is weakened and unable to hold in check the usual numbers of invading organisms? Does the bactericidal substance of the udder have a selective action on bacteria, or does it have the same effect on all strains? Are the normal udder bacteria able to increase in virulence under certain circumstances? Until such questions can be answered, it is unlikely that mastitis infection will be efficiently controlled.

5. Relation of Human Pathogens to Mastitis.

The organisms most commonly associated with bovine mastitis are non-pathogenic to man. However, several human pathogens have been found from time to time to cause mastitis.

The most frequently encountered of these is Str. pyogenes. Numerous references can be found in the literature to epidemics of scarlet fever and septic sore throat which have been traced to cases of mastitis due to Str. pyogenes. The patients had consumed unpasteurized milk or milk products containing the secretion from the infected cows. In almost every case it has been shown that the animal became infected from a human carrier. Stebbins et al (104) reported on 7 milk-borne epidemics in New York State from 1934-1936, involving 3 epidemics of scarlet fever with 806 cases and 16 deaths, and 4 epidemics of septic sore throat with 723 cases

and 8 deaths. Six of the seven epidemics were traced to cows suffering from acute mastitis and harbouring streptococci indistinguishable from those isolated from the patients.

Certain staphylococci are able to produce an enterotoxin which causes acute gastro-enteritis in man. Staphylococci of this type have been incriminated in certain cases of mastitis (24). These enterotoxin-producing staphylococci may infect the udder from man or from primary bovine infections. It should be noted in this connection that this toxin is heat stable and that if milk, containing toxin-producing staphylococci, is held at temperatures high enough for the organisms to multiply and produce toxin, subsequent pasteurization of the milk will not destroy the toxin. Crabtree and Litterer (27) reported a series of 242 attacks of severe gastro-enteritis in 97 persons which was traced to two mastitic cows harbouring toxin-producing staphylococci in a herd supplying raw milk to the infected persons.

Cone (26) reports that Ps. aeruginosa has been suspected of causing a type of dysentery in man. Although there is as yet no proof that such is the case, the source of the causative organism could easily be mastitic milk from cows infected with Ps. aeruginosa.

Man usually acquires the human pathogens of mastitis origin by drinking unpasteurized milk. It appears, however, that products made from raw milk may also harbour these organisms. Bryan and Bryan (13) found that a strain of Str.

agalactiae, a strain of Str. pyogenes, and a hemolytic and a non-hemolytic strain of Staph. aureus survived for 6 months in both salted and unsalted butter made from sweet and ripened unpasteurized cream. Yale and Marquardt (113) observed that Str. pyogenes survived over 18 weeks in cheddar cheese ripened at 45°F. and 9-11 weeks in cheese cured at 62°F. They concluded that the survival of pathogens in cheese was dependent on the kind of cheese, pH, moisture content, salt content, and curing temperature. Despite these findings, there are no records of streptococcic epidemics ever having been traced to cheese.

The importance of using only pasteurized milk and milk products is to be especially emphasized when it is considered that, in many instances, human pathogens cause subclinical mastitis which cannot be readily detected.

Factors Predisposing the Udder to Mastitis Infection.

Since most of the organisms which commonly produce mastitis are of low invasive power, and since they appear to be present in the environment of the healthy udder without producing disease, it follows that there must be factors other than bacterial invasion which are essential for the initiation of infection. These other factors have, in fact, come to be considered by many workers to be of greater importance than the organisms themselves.

1. Breed.

Many surveys have been made to determine whether certain breeds of cattle are more disposed to mastitis infection than others. However, no proof has been forthcoming that mastitis infection is more prevalent in any particular breed than in any other. The apparent higher incidence of the disease in certain breeds as reported by different workers may be explained by the fact that the breed in question comprised a greater percentage of the cow population studied. Murphy (76) found that the teat mucous membranes of Holsteins were more pocketed than those of Guernseys. Contrary to what would be expected, however, he did not find that the incidence of mastitis was related to the amount of pocketing.

2. Inheritance.

The inheritance of susceptibility to mastitis has been suspected as a contributary factor to infection. The effect of inheritance is probably of an indirect nature. As a result of the present-day practice of breeding cows for greater milk production, it is to be suspected that in the high producing cow the udder tissue is placed under such an extreme working pressure that its normal metabolism is upset, and so the animal is less able to resist bacterial invasion. Also, high producing cows, especially of the Holstein breed, often have large pendulous udders which are readily injured.

Murphy et al (79) made a study of the mastitis records of two families of cows and concluded that heredity played a part in susceptibility to mastitis in the animals studied. Fincher (37) suggests that bulls improperly selected from dams with weak udders may cause serious amounts of mastitis through genetic influences. Some workers believe that the internal structure of the teat, that is, the size of the streak canal and the amount of pocketing, which may be an inheritable factor, is related to the lodgement of bacteria and their gaining a foothold in the udder (59).

3. Stage of Lactation.

Acute mastitis due to staphylococci frequently occurs immediately after calving. It is probable that these organisms are opportunists and take advantage of the congestion in the udder at freshening time to establish themselves. Little and Foley (66) attribute the greater incidence of staphylococcic mastitis in newly freshened cows to the presence of increased amounts of blood serum in the milk at this time. In in vitro studies, they found that the presence of serum overcame the bacteriostatic action of milk on staphylococci, and concluded that the high serum content of colostrum was conducive to rapid multiplication of staphylococci in the udder.

"Summer mastitis" caused by C. pyogenes appears to be most prevalent in dry cows. As discussed previously,

this is likely due to a combination of factors such as cows sucking one another, and flies feeding on the secretion left on the ends of the teats after sucking. These conditions apparently favour the development of this bacterium without stimulating the activity of other mastitic organisms.

With the common chronic form of the disease, there does not appear to be any particular stage in the lactation period when the animal is most susceptible to infection. Because the disease is slow in its development, the first visible signs of infection are not likely to appear until late in the lactation period. The initial infection, however, undoubtedly took place much earlier.

4. Age of Cow.

It is to be expected that the older the cow, the greater is the likelihood of its having mastitis. As the number of lactations increases, the udder tissue becomes exhausted and the teat muscles relaxed, and so the animal is less able to resist invading organisms. Especially is this so under present-day high production practices. The chronic nature of the common form of the disease also helps to explain why mastitis is more prevalent in older cows. Bryan (11) found that the majority of cows of eight years of age or older were infected with streptococcic mastitis.

Many workers have become alarmed at finding mastitic streptococci in the milk of first-calf heifers and in

the mammary tissue of virgin heifers (51). They do not, however, report whether there were any signs of infection other than the presence of mastitic organisms. The bacteria probably reached the udder through a prematurely opened streak canal from calves sucking one another after drinking mastitic milk. In such instances it is likely that the organisms are living in the udder in a quiescent state, not causing any infection, but ready when suitable conditions arise to produce disease. In many cases where Str. agalactiae has been recovered from the milk of first-calf heifers during the first few days after freshening, the organism has later disappeared from the milk.

5. Completeness of Milking.

The effect of incomplete milking on the development of mastitis is not well agreed upon by the various workers in this field. Some investigators believe that when quantities of milk are left in the gland after milking, the bacteria present in the udder multiply rapidly on this excellent food supply, and so are able to initiate infection. When it is recalled, however, that milk secretion is a continuous process, and that the udder is never completely free of milk, the validity of the above theory may be questioned. Schalm and Mead (97) found that incomplete milking had little effect on the physical appearance of milk from healthy quarters but that it appeared to aggravate the symptoms of mas-

titis due to Str. agalactiae. They also found that frequent milking relieved the symptoms of mastitis caused by Str. agalactiae.

In acute cases of mastitis, frequent milking of the affected quarter is usually recommended. The purpose of such a procedure is to eliminate as many organisms as possible from the udder and to reduce the intramammary pressure. Some workers advise the cessation of milking as an aid in curing mastitis. They claim that by so doing the secretory cells of the udder are relieved of the duty of milk secretion, and the udder tissues can then concentrate on overcoming the infecting bacteria.

When the practice of overstocking is followed to induce the rapid drying up of late lactation cows, the amount of milk left in the udder is probably much greater than that which would remain as a result of careless milking or of not stripping after machine milking. In such cases, the leaving of such large quantities of milk in the udder is undoubtedly conducive to congestion and bacterial multiplication.

6. Size of Herd.

In practice, it has been found that mastitis is more prevalent in larger herds than in smaller ones. This may be readily explained. In small herds the owner frequently looks after the complete herd himself, and consequently readily detects the earliest signs of infection. Although

there are usually better sanitary facilities in large herds, because of the increased number of animals, there are more attendants, with the result that less individual attention is given each cow. Also, milking machines are more frequently used in large herds, and so flakiness in the fore-milk, which is often one of the first signs of a flare-up of chronic mastitis, may not be detected. Another important consideration is that in small herds replacement animals are usually raised on the farm, whereas in large herds the number of cows in the herd is maintained by the purchase of mature cows which are possible sources of mastitis. Bryan has shown (11) that under identical conditions of management, there is no difference in the incidence of mastitis in large and small herds.

7. Milking Machines.

The milking machine has frequently been incriminated as an important factor in the establishment of mastitis infection in the udder. If properly used, the milking machine should be a definite aid to the farmer in maintaining maximum milk production. In view of the fact that the let down of milk is dependent on the secretion of oxytocin by the pituitary gland, and that the amount of this hormone in the blood is rapidly depleted, rapid milking as can best be accomplished by the use of the milking machine should increase the amount of milk produced per cow per milking over that obtained by hand milking.

It is the careless use of the milking machine that is conducive to the development of mastitis. When the teat cups are not properly sterilized between cows, they may spread mastitic organisms from diseased to healthy cows. Too high a vacume, too rapid or uneven pulsation, letting teat cups ride too high on the teats, leaving the machine on too long, all produce strain on the udder tissue, and if carried on for any length of time may predispose the udder to mastitis infection.

Numerous workers have tried to prove that a particular brand of milking machine is more contributory to mastitis infection than another. Because of the complexity of such a problem and of the number of factors to be considered, the results have not been very satisfactory.

8. Season and Weather.

The effect of the season of the year and of weather conditions on the incidence of mastitis is not readily studied because of the number of factors involved. It is known, however, that sudden chilling is conducive to mastitis infection. It is likely that a non-infectious condition is first set up in the udder and that bacterial invasion follows.

9. Feed.

The relation of feeding practices to mastitis infection is probably of an indirect nature. In trying to obtain maximum milk production, the farmer keeps his cows on a

high protein ration. It is likely the effect of the high milk production in weakening the udder and not the feed itself which contributes to the production of mastitis.

Of interest in this connection is the report of Wiedmann (112) that mastitis is due to a deficiency of carbohydrate in the diet. However, Wesche (110), working on the same problem was unable to duplicate Wiedmann's results.

10. Trauma.

It is generally agreed that the most important factor predisposing the udder to mastitis infection is injury. This may entail injury to the udder itself by bruising from high door sills, stumps, etc., by kicking, horning, and by penetration of the udder tissue by barbed wire. More frequently it entails injury to the teats especially from being stepped on. Teat injuries allow bacteria to enter the udder more easily. The point of injury acts as a focus permitting the bacteria to gain a foothold in the udder. Further, the defensive and regenerative mechanisms of the udder are occupied in repairing the injury and are less able to combat the invading organisms.

11. Other Infectious Diseases.

As mentioned previously, it is likely that Br. abortus lesions in the udder predispose to mastitis infection. Vaccinia virus and the virus of foot and mouth disease produce lesions on the teats and it is thought that, on occasion,

these might act as foci for invading bacteria.

An interesting observation relative to the effect of other bacteria on the development of mastitic organisms, is the report of Pounden and Johnson (86) that an udder staphylococcus enhanced the acid produced in Hotis tests by mastitic streptococci. It might be of value in understanding the relative activities of mastitic organisms in the mammary gland to know whether or not a similar stimulation could take place in the udder.

Extent of Mastitis Infection.

Because of the multiplicity of ways in which the disease manifests itself, and because of the variations in the extent of the changes produced in the udder tissue and in the alterations in the composition of the milk, it is very difficult to define precisely what state of the udder and its secretion denotes the dividing line between a healthy and a mastitic condition. In evaluating the literature, it is important to note on what basis the various workers determine whether or not an animal is mastitic. In most cases, abnormalities in the composition of the milk can be detected earlier in the course of the disease than can changes in the udder tissue. Organisms capable of causing mastitis may be isolated from milk normal in chemical composition and from non-indurated udders. On the other hand, it is sometimes impossible to isolate organisms from quarters obviously dis-

eased as determined by other tests. Thus in interpreting studies on the extent to which herds are infected with mastitis, it is important to take into consideration the methods of diagnosis, since, in general, a bacteriological examination of the milk reveals more positive cases than a chemical examination, and the latter indicates a greater number of diseased cows than a physical examination of the udder.

Gwatkin et al (42) in a study of 594 cows in 28 herds in Ontario found 39% of the cows positive for mastitis and 9.6% suspicious. They based their conclusions on the following tests: physical examination of the udder, appearance of the milk, brom thymol blue reaction, rennet coagulation test, microscopic and bacteriological examination of quarter samples of milk. The percent of infection in individual herds ranged from 15-91. In a further study of 265 positive cases, including those considered above, they found that 29.8% of the animals were infected in one quarter only, 21.9% in two quarters, 16.6% in three quarters and 31.7% in all four quarters. When considered entirely on a quarter basis, this study revealed that 62.5% of the total number of quarters of the 265 positive cows were infected. If the 362 negative and suspicious animals from their first study are added to these 265 positive cows, and the results again considered on a quarter basis, only 26.4% of the total number of quarters of the 627 cows are found to be infected.

Rosell (87) in a five year study in Quebec found an

average of 34.6% of the 1,838 cows studied to be positive for mastitis. In individual herds the percent of cows infected varied from 19-97. On quarter samples of milk he determined the catalase content, percent chlorine, pH with brom thymol blue, and amount of sediment in the milk, and made a microscopic examination of the sediment. Rosell concluded from his studies that there were very few herds which, after adequate testing, were completely free from mastitis.

Bryan (11) made a study of 2,715 cows in 322 herds in Michigan, using a leucocyte count of over one million per c.c. together with the presence of streptococci in incubated composite milk samples as his criterion of infection. 26.2% of the cows studied were found to have streptococcic mastitis and only 14% of the herds were free from infection.

In England, Edwards (33) examined 809 cows in 18 herds and found 36.4% of the cows to be infected in at least one quarter with streptococcic mastitis, as revealed by a cultural examination of the sediment from quarter samples of milk on Edwards' aesculin crystal violet medium (32). The incidence in individual herds varied from 10-84%. In a detailed study of 218 infected cows, Edwards found that 36.2% of the positive cows were infected in one quarter only, 27.5% in two quarters, 15.6% in three quarters and 20.7% in all four quarters. 55.16% of the total number of quarters of the infected animals were positive for streptococcic mastitis. On including the 514 negative cows from the first study with these 218

positive cows, and considering the results on a quarter basis, 16.4% of the total number of quarters were found to be positive.

The findings of these workers indicate that in any representative group of cows, about 25-40% of the animals can be expected to be infected with mastitis, and that about 20% of the quarters of the total number of cows will be positive for this disease. In individual herds the disease may be anticipated to involve approximately 10 to over 90% of the animals.

Effect of Mastitis on Milk Yield.

It is a well known fact that mastitis reduces the amount of milk produced by infected animals. The extent of this reduction depends on the number of quarters involved and on the seriousness of the condition in each quarter. It reaches its ultimate when the affected quarter is completely atrophied and secretes no milk at all. Because the amount of milk produced even by normal cows is such a variable quantity and depends on so many factors, it is very difficult to determine the reduction in milk yield brought about by sub-clinical mastitis.

Among the first to work on this problem were Shaw and Beam (101), who found that quarters, positive to the brom thymol blue, percent chlorine, cell count and catalase tests, produced approximately 22% less milk than the opposite non-

infected quarters, after the maximum variation between the yields from non-infected quarters had been allowed for. It is important to note, however, that the results were computed by the difference in yield between the infected quarter and the opposite non-infected one. Thus, if the inefficiency of one quarter had stimulated the other three to greater than normal milk production, as appears to be the case where a single or at the most two quarters are infected, this compensatory action would tend to increase the apparent differences between the yields of infected and non-infected quarters.

Minett and Martin (73) made a study of two herds of Ayrshire and Friesian cows over a total of 373 lactation periods. Both herds were infected with sub-clinical mastitis due to Str. agalactiae as determined by a bacteriological examination of the sediment from quarter samples of milk. The total milk production of each cow for an entire lactation period was corrected for age, length of dry period and month of calving, and the corrected results were subject to statistical analysis. An average reduction in milk yield of 954 pounds per lactation was found for the infected cows studied.

White et al (111) studied 35 cows over a total of 198 lactation periods. On comparing the results obtained from negative cows with those from the same cows after becoming infected with sub-clinical mastitis as determined by the brom thymol blue test, sediment test, leucocyte count and bacteriological examination of the milk, they found that when

only one quarter of the udder was affected, there was no decrease in the milk yield of the cow. A reduction in yield takes place, however, when two quarters are involved, and this reduction increases with each additional quarter infected, until when all four quarters are involved milk production is decreased by about 15-20%.

Hucker et al (53) in a study of the milk production records of 35 cows over 3 lactation periods concluded that when the only evidence of mastitis was a leucocyte count of over 500,000 per c.c., or the presence of streptococci, there was no reduction in milk yield. The yield was not affected until the disease had progressed to the point where the foremilk was alkaline to brom thymol blue or the physical appearance of the milk was grossly altered. They also concluded that a mild infection of all four quarters had about the same effect on milk production as a more severe infection of one quarter only.

In the early stages of chronic mastitis, the milk yield is undoubtedly affected very little if at all. As the disease progresses, more secretory cells are destroyed and the amount of milk produced by the affected quarter diminishes accordingly. In cases of sub-clinical mastitis it is not likely that the reduction in the milk yield is very great, but when the potential milk production of a large number of such infected cows is considered, the reduction of yield assumes considerable economic importance.

Effect of Mastitis on the Composition of Milk.

The effect of mastitis infection on the chemical composition and physical properties of milk have been discussed at length by Rosell (88). In mastitis, the fat content of the milk is usually lowered. The solids-not-fat content is also decreased. The calcium and potassium contents are diminished, but the amounts of sodium and chlorine are increased. Lactose and casein are reduced but albumin and the non-protein nitrogen fraction increase. The pH is generally more alkaline than usual. The electrical conductivity is increased due to the greater salt content. The viscosity of the milk, on the other hand, is decreased.

Milk from mastitic animals usually shows a reduced stability to heat. This is probably due to its decreased calcium content and increased albumin content and possibly also to its high pH. Welch and Doan (109) found that when mastitic milk was mixed with normal milk and the resulting mixture concentrated to a solids content comparable to that of condensed milk, the mastitic milk tended to render the normal milk less stable towards heat. It can thus be seen that if appreciable quantities of milk from mastitic animals are used in the manufacture of evaporated and condensed milk, serious difficulties may be encountered in the sterilization of these products.

As a rule, mastitic milk takes longer to coagulate with rennet than normal milk and the resulting curd is not so firm (103). This is probably related to the decreased

amounts of casein and calcium and to the high pH in mastitic milk. Johns et al (57) found that the yield of cheddar cheese was reduced when milk from cows with sub-clinical mastitis was used. They did not, however, find the quality of the cheese to be affected. Mastitic milk, because of its low curd tension, tends to produce weak-bodied cheese. If milk from cows more seriously infected than those studied by Johns et al were used to make cheese, it is very likely that the flavour and body of the resulting cheese would be affected (54).

The low calcium, casein and lactose content of mastitic milk renders it inferior to normal milk as a source of these substances for human nutrition. In view of the prevalence of mastitis among the dairy cow population, it is probable that these deficiencies have not received the attention warranted. Another consideration in this connection is the report of Schönberg (98) that milk from mastitis-infected cows is deficient in riboflavin.

The extent of the chemical alteration in the milk of mastitic animals is related to the severity of the disease - the more advanced the case the greater is the alteration. Thus the effect of the abnormal chemical composition of mastitic milk on processed milk and on human nutrition is dependent on the severity of the infection in the individual cow, and also on the amount of abnormal milk in the total milk supply.

EXPERIMENTAL.

Outline of Research Council Program.

The work undertaken by the Research Council on mastitis has as its objective the lessening of the incidence of this disease in the dairy herds of British Columbia. Throughout the course of this study, major emphasis has been placed on the following aspects of the problem, which are discussed under separate headings:

1. a survey of the extent of mastitis infection in British Columbia.

2. a study of the factors predisposing the udder to mastitis infection.

3. an evaluation of the tests used for the detection of mastitis.

4. a detailed study of the organisms associated with mastitis and an evaluation of the methods used for their classification.

5. a study of the methods used for the control of the spread of mastitis infection.

6. a study of the treatments used for mastitis.

The experimental data to be discussed under the above headings have been obtained from three sources:

1. a preliminary study of the extent of mastitis infection in the North Okanagan Valley which has been designated as the "North Okanagan Study".

2. a general survey of the incidence of the disease in British Columbia which has been called the "General Survey".

3. a detailed study of four experimental herds, established for the purpose of determining the efficiency of a management program for the control of the disease. This project has been referred to as the "Experimental Herd Study".

Under some headings, the results from all three sources are discussed, under others, only those from one or two. In all cases, however, the data from each of the three projects are treated separately.

General Methods.

1. North Okanagan Study.

During the course of a study of some of the problems associated with cheese-making in the North Okanagan Valley, an examination was made of the milk supply of a cheese factory in that area, employing the methylene blue reductase test, the resazurin test, and a semi-quantitative Breed count on the bulk milk from each shipper. Following this study, nine herds were selected on the basis of the rapid partial reduction and slow complete reduction of methylene blue and resazurin, and of the presence of more than the average number of leucocytes and of chaining cocci in the bulk herd milk. These herds were then subjected to analysis for mastitis.

Milk samples were taken as aseptically as possible into sterile sample bottles. In each case, about 40 c.c. of first strippings from each quarter of the udder were obtained. The samples were taken at the evening milking, left overnight at room temperature and tested the next morning. The following tests for mastitis were performed on the samples: physical appearance of the milk, Geneva blotter test, modified Whiteside test, pH determination with brom cresol purple, Hotis test, percent chlorine, leucocyte count and blood agar plate technique.

2. General Survey.

The general survey of the extent of mastitis infection in British Columbia was made possible by the co-operation of interested practising veterinarians throughout the province. These veterinarians undertook the responsibility of collecting milk samples from suspicious and positive cows and of obtaining field data relevant to the case being studied and to the general problem of mastitis control.

As required, the veterinarian was supplied with packages containing four sterile screw-cap, 1 1/2 oz., bottles for the collection of milk samples, to be forwarded to the laboratory for examination. He was also supplied with a field note book for the collection of data regarding the case history of the cow under study and related information. This data was to be sent to the laboratory with the milk samples.

A copy of the data sheet from the field note book is appended hereto as Exhibit "A". The field note book also provided for the recording of observations concerning the history of the case subsequent to the taking of the initial milk samples. A copy of this history sheet is appended as Exhibit "B".

Milk samples were taken from clinically positive and suspicious animals and occasionally from negative cows in highly infected herds. Quarter samples were to be taken from all four quarters of the udder, even though every quarter was not necessarily infected. In so far as was possible, first strippings were to be taken. The samples were sent to the laboratory by mail or express. Icing of the samples in transit was not carried out. The samples were tested as soon as possible after reaching the laboratory, and the results of the laboratory diagnosis were sent to the veterinarian as expeditiously as possible, either by mail or telephone, in order to assist him in prescribing treatment measures. The following laboratory tests were employed for the detection of mastitis: physical examination of the milk, modified Whiteside test, pH determination with brom cresol purple, Hotis test, percent chlorine, leucocyte count, and blood agar plate technique.

3. Experimental Herd Study.

In order to study the effect of a strict manage-

EXHIBIT "A".

DATA SHEET FOR THE COLLECTION OF FIELD DATA RELEVANT TO THE CASE UNDER STUDY.

MASTITIS RESEARCH PROJECT

Doctor.....

Date .....

Herd Owner .....

Address .....

Cow Ident. { Tattoo or Ear Tag..... Breed..... Age.....

Date of Calving..... Daily Milk Production.....

Type of Mastitis..... Duration of Attack.....

Case History.....

.....

Herd History.....

.....

Daily Milkings: { A.M..... P.M.....
A.M..... P.M.....

How Milked .....

Machine:- Name of Mfgr..... Type of Unit.....

Feeding Methods .....

Percent Protein.....

Supplements Used .....

Sanitary Methods .....

.....

Treatment .....

.....

Milk Samples for Lab.: RF.....LF.....RH.....LH.....

Time.....First Stripping: Yes.....No.....

Please keep record of Treatment and Results on Page 3.

EXHIBIT "B".

HISTORY SHEET FOR THE COLLECTION OF DATA REGARDING THE CASE HISTORY SUBSEQUENT TO THE TAKING OF THE INITIAL MILK SAMPLES.

Herd Owner.....

Address .....

Cow Ident. { Tattoo or Ear Tag..... Breed..... Age.....

Condition of Udder.....

Treatment Used .....

Repeated .....

.....

Clinical Notes .....

.....

.....

.....

.....

.....

.....

.....

.....

.....

Results Obtained .....

.....

Recovered Condition of Udder.....

.....

ment program on the control of mastitis, four herds, known to have a high incidence of mastitis, were selected in different parts of the Lower Fraser Valley. A legal agreement outlining the duties and responsibilities of the Research Council, the veterinarian in charge of each herd, and the co-operating farmer was drawn up. The Council assumed the responsibility for the conduct of the research project. The veterinarian was made responsible for the field work required for the program, and had charge of the supervision of the milking practices to be followed by the co-operating farmer, as outlined in the schedule to the agreement which is given below. The herd owner undertook to give the veterinarian access to his herd at all times, and agreed to carry out the herd management and milking procedures as prescribed. The farmer also assumed the responsibility of reporting all cases of mastitis attacks immediately to the veterinarian, so that there would be the closest liason between the administration of treatment by the veterinarian, and the work of the laboratory on milk samples procured from the diseased animals.

SCHEDULE.

TO THE AGREEMENT ENTERED INTO
BY THE RESEARCH COUNCIL, THE
VETERINARIAN, AND THE HERD OWNER.

Outline of Herd Management Practices to be Carried Out
By all Co-operating Dairy Farm Owners

A. GENERAL SANITATION
Barns, paddocks and pasture fields used by milking cows

to be kept in good general condition. Fences in good repair - no free wire, hanging loose. Fields free of protuding objects, stumps, roots, logs, brush and stone upon which udder injuries could occur. Stalls and stanchions to be kept in good repair to avoid all udder injuries.

B. STABLE SANITATION
Stable to be kept clean, bright and airy. Walls and ceilings to be cleaned once yearly. Stalls and gutters to be washed daily with running water or washed down once each week with lye solution, 1 lb. to 20 qts. water. Bedding - all stables to be kept well bedded with clean, dry straw or other material.

C. FEEDING
Feed well balanced rations plus necessary mineral and vitamin supplements when indicated. Protein in ration not to exceed 16%. In case of mastitis flare-up or acute attack in any cow or cows, grain rations for such animal to be reduced at once, and steps taken to isolate such animals from milking line.

D. TREATMENT OF MASTITIS CASES
Udder injuries or teat injuries to be handled only at and with the advice of the co-operating Veterinarian. Under no circumstances are milking tubes, teat bogies or dilators to be used except by and with the advice of the Veterinarian.

E. REPLACEMENT COWS
Owners are strongly advised to raise, insofar as possible, their replacement requirements. Cows or in-calf heifers purchased for replacement are to be isolated from the milking for a period of thirty days and to have passed two clean tests for Mastitis before being admitted to the milking line of cows.

F. CALF REARING
Owners are requested to raise all heifer calves on milk known to be from healthy, Mastitis-free cows. Milk from Mastitis-infected cows should be pasteurized or boiled for one minute before being fed to any livestock.

G. RECOMMENDATIONS FOR MILKING PROGRAMME
(1) Have a preliminary test made for Mastitis on every cow milking on the farm.
(ii) Arrange cows in milking order in the dairy barn in such a manner that all clean cows are milked in Group 1, suspicious or questionable cows milked as Group 2, and Mastitic or infected cows as Group 3.

H. EQUIPMENT REQUIRED

Supplementary

(i) One wiping cloth for each cow in milking herd (unbleached cotton 18" square or 12" x 24" recommended).

(ii) Four sanitary pails to supplement milking equipment:
Pail No. 1 - Chlorine Solution (250 ppm.) to be kept as near 130°F. as possible. All towels for udder washing to be kept in this solution.
Pail No. 2 - Empty pail to receive used towels as each udder is washed.
Pail No. 3 - Clean, cold water to rinse milking cups as removed from each cow.
Pail No. 4 - Warm chlorine solution (250 ppm.) to immerse and hold milking cups between each cow being milked.

(iii) Strip cup and black screen of fine mesh to be used as a check test on first strippings immediately before applying milking unit.

(iv) Light weight wheeled truck or suspended carrier to accommodate above equipment and milking pails - carried well above spotter level in milking alley.

(v) Dipping Cup - small cup of chlorine solution to be used to dip each teat of the milked cow as soon as cows have been given final check stripping. (NOTE: The chlorine solution in cup to be discarded after each cow.)

Milking Equipment

(i) If machine - two units per operator is recommended.

(ii) One extra milking pail with lid per unit in operations is essential so that no pouring of milk from pail to other containers be required at the milking line.

(iii) Milking scales and recording sheets.

Routine of Milking

(i) Prepare all equipment, chlorine solutions, washing cloths and strip cups in readiness on movable platform.

(ii) Check temperature of chlorine solution and renew if temperature drops 130° to 100°F. unless some arrangement can be made to maintain heat.

(iii) Wash the udder carefully and completely. Ring towel out and wipe dry. Discard towel into empty pail.

(iv) Using strip cup, draw one or two full hand squeezes of milk from each quarter in rotation through screen and note character of secretion. If clear of all flakes, apply machine.

(v) Apply milking machine for three to four minutes.

Watch closely to remove as soon as cow is milked out. Remove machine promptly.

(vi) Rinse teat cups in cold water and then immerse in chlorine solution.

(vii) Stripping cow - This should be done immediately after machine is removed. Use only full hand squeezes and avoid excessive stripping.

(viii) Use dipping cup - on all four quarters in rotation taking a fresh cup of chlorine solution and discarding it after treatment of each individual cow.

NOTE: If two men are available at milking, it is advisable to divide the work as follows:

One man to wash udders, do first stripping test and operate milking machine. Second man to finish milking by final stripping, complete the operation by application of chlorine solution with dipping cup to each teat and weigh and record the milk for each individual cow.

I. GENERAL

Close application of the above principles will materially assist in preventing Mastitis in your herd. It will assist you in eliminating the disease promptly by knowing all infected cows; and in general routine practice it will speed up the milking operation so as to permit its completion in a clean, sanitary manner that will pay dividends.

Complete co-operation, exchange of important observations as between the owner, the veterinarian and the laboratory workers will go far towards the ultimate objective of the complete control of Mastitis in British Columbia dairy herds.

- - - - - -

Milk samples were taken from all cows in the experimental herds at frequent intervals and tested in the laboratory for chemical abnormality and for the presence of mastitis organisms. Physical examinations of the udders of all cows were to be made regularly by the veterinarian in charge of each herd. In addition, any and all treatments used for mastitis in these herds, and the results of such treatments,

were to be recorded by the veterinarian.

It was planned to carry on the experimental herd study for at least two or three years, and so the results reported herein on this project are to be considered as being in the nature of a progress report. The data from this study in the present report cover the first two routine tests on the four herds, the second test being performed approximately three months after the first. In addition, two of the herds (#1 and #2) were each tested once before the experimental herd study was initiated. These tests were made about two months before the first general test on all herds. The data from these two examinations are included in this section of the report.

When an entire herd was tested at one time, the milk samples were taken by the laboratory personnel. The udders and teats were carefully washed with a warm chlorine solution before the samples were obtained. For the first part of the work, the samples consisted of the very first milk obtained from a quarter. For the last series of tests, however, two streams of milk were discarded from each quarter before the samples were collected. It was hoped by this procedure to eliminate most of the contaminating organisms which may have reached the lower part of the udder through the teat canal during the interval between milkings. In the first testing of the four herds, quarter samples of milk were taken from all cows. In the second test, composite samples were obtained

from those animals in herds #1 and #4 which were found free from evidence of mastitis at the first testing.

The samples were taken at the evening milking, left at room temperature over night and tested the next day. The same tests for mastitis were employed as in the general survey, namely, physical examination of the milk, modified Whiteside test, pH determination with brom cresol purple, Hotis test, percent chlorine, leucocyte count and blood agar plate technique. At the first general testing, representative colonies were picked from the plates of all samples taken from the cows of herd #1 and the cultures so obtained were subjected to detailed study. At the second testing a similar study was carried out on organisms isolated from the milk of the animals in herd #2.

Methods of Clinical Diagnosis.

In the case of acute mastitis, the symptoms of the disease are readily observed. With sub-clinical mastitis, however, the following procedures must be used in order to detect lesions in the udder: the examination of the teat for patency and fibrosis, the examination of the udder for symmetry and balance, and the palpation of the milked-out udder for the extent of induration and fibrosis. These methods are described in detail and their usefulness discussed by Udall and Johnson (106).

Methods of Laboratory Diagnosis.

The methods used for the laboratory diagnosis of mastitis were altered slightly from time to time throughout the study as improvements suggested themselves. An outline of the various procedures and the changes made in them is given below.

1. Physical Appearance of the Milk.

All milk samples were subjected to a visual examination for the presence of flakes, clots, serum, blood, etc.

2. Modified Whiteside Test.

The modified Whiteside test was carried out on all samples according to the method of Murphy and Hanson (77). 5 loopfuls of milk were placed on a microscope slide and 1-2 loopfuls of 1 N sodium hydroxide were mixed with them for about 40 seconds. The results which are best observed against a dark background, are interpreted as follows:

0 reaction: uniform opaque fluid.

$\pm$ reaction: similar to the 0 reaction with the addition of a few very small white flakes.

1+ reaction: similar to $\pm$ reaction but with more and larger flakes.

2+ reaction: clear background with large white flakes.

3+ reaction: clear background with large flakes tending to clump together.

4+ reaction: viscid mass lifting off the slide with

the loop.

3. Geneva Blotter Test.

The Geneva blotter test is a test for pH which has been adapted for use in the field. It consists of a piece of blotting paper with four small areas impregnated with brom thymol blue on which a drop or two of milk from each quarter is placed. Although this test was designed for use in the barn, it was employed on all samples in the North Okanagan study, 1 or 2 drops of milk being applied to each area of dye.

4. pH Determination with Brom Cresol Purple.

The determinatiom of pH was carried out in conjunction with the Hotis test. 9.5 c.c. of milk were placed in a sterile rubber-stoppered test tube. 0.5 c.c. of a 0.5% aqueous solution of brom cresol purple were added to each tube. The tubes were then shaken and the colour of the milk observed. The colour reactions are interpreted as follows:

normal:	greyish mauve colour.
very slightly alkaline:) slightly alkaline:) alkaline:)	increasing shades of purple.
very alkaline:	deep purple colour.
very slightly acid:) slightly acid:) acid:)	increasing shades of yellow.
very acid:	bright canary yellow colour.

During part of the work, a brom cresol purple colour standard was employed. This standard was, however, not completely satisfactory, owing to the differences obtained in the depth of colour with milks of different fat content. Of necessity, the standard was prepared from skim milk. Milks from

Jersey and Guernsey cows tend to give a lighter, more bluish shade than the standard, whereas milks from Ayrshire and Holstein cows give shades of colour more approaching those of the standard. Thus the use of the standard for high fat content milks would tend to grade these samples as more normal in pH than should actually be the case. For most of the samples tested, however, no standard was used, and it was found relatively easy, after a little experience, to distinquish between the various shades of colour, especially if a fairly large number of samples were examined at one time.

5. Hotis Test.

The procedure of Hotis and Miller (49) was used in carrying out the Hotis test. The tubes used for the pH determination were incubated at 37°C. and examined after 24 and 48 hours for colour change, presence of flakes along the sides and bottom of the tube, degree of clotting and digestion of the milk. Unfortunately, in the North Okanagan study, the results of this test were very unsatisfactory. Owing to inexperience at the time in interpreting the data obtained by this test, the results of the Hotis test from the North Okanagan study are not recorded in the tables. Positive findings, however, were used as confirmatory evidence in classifying the quarters.

6. Percent Chlorine.

Rosell's method (87) for determining the chlorine

content of milk was used throughout the study. The test was carried out as follows: 40 c.c. of distilled water, 10 c.c. of milk and 8 drops of a 10% aqueous solution of potassium chromate were placed in a flask. N/10 silver nitrate was titrated into the above solution until the first permanent colour change was noted. The percent of chlorine in the milk is obtained by multiplying the number of c.c. of $AgNO_3$ required by 0.0355. Owing to an insufficient supply of silver nitrate, only about half of the samples taken in the North Okanagan study could be examined by this test.

7. Leucocyte Count.

The leucocyte count was carried out by a modification of the Breed technique for counting the bacteria in milk. Smears were made by spreading a 2 mm. loopful (I.D.) of milk over 1 sq. cm. area on a microscope slide and were stained by the Newman method (80). The number of leucocytes in 30 fields were counted with a calibrated microscope, and the results calculated to give the cell count per c.c. of milk. It should be noted, however, that the quantity of milk delivered by the 2 mm. loop was slightly less than the 0.01 c.c. employed for the standard Breed technique. Thus the counts recorded herein may be slightly lower than those obtained by other workers using the standard method.

In the North Okanagan study and the general survey, the leucocyte count was performed on the fresh milk samples.

In the experimental herd study, however, the samples, after the pH, percent chlorine and Whiteside tests had been performed, were incubated at 37°C. for 16-24 hours, and the leucocyte smears made after incubation. By this procedure, the bacteria in the sample, especially the streptococci, are given a chance to multiply and so should be more readily detected on microscopic examination of the incubated milk. Since carefully taken milk samples do not usually clot during this period of incubation, the procedure does not interfere with the preparation of a uniform smear for the leucocyte count, and, in addition, useful information is obtained regarding the types of organisms present. The samples studied in the general survey were frequently two or three days old on reaching the laboratory, and so it was deemed inadvisable to incubate them before making the smears. However, in some cases in the general survey, the leucocyte count was performed on the unincubated sample, the sample was then incubated for 16-24 hours, and another smear was made to study the types of organisms which may have developed during incubation.

8. Microscopic Study of the Organisms Present in the Milk Samples.

The relative numbers and kinds of bacteria present in the smears, made to determine the leucocyte count of the milk samples, were noted. The smears were always carefully examined for short and long chaining streptococci.

9. Cultural Study of the Organisms Present in the Milk Samples.

The techniques used for the preparation of blood agar plates from the milk samples were altered a number of times during the course of the study.

Several tryptose agar bases were used in the early part of the work for making the blood agar, and it was found that most of these media gave comparable results. The medium finally selected and used for the greater part of the study was Difco tryptose blood agar base which has the following composition:

beef extract:	0.3%
tryptose:	1.0%
sodium chloride:	0.5%
agar:	1.7%

For part of the general survey and for all of the experimental herd study, the milks were plated on Edwards' crystal violet aesculin agar (32) as well as on the tryptose blood agar. Edwards' medium is designed to facilitate the differentiation of streptococci by suppressing the growth of other organisms while permitting the streptococci to develop normally. At first, this medium was prepared as directed by Edwards with nutrient agar, crystal violet and aesculin. It is known, however, that some streptococci do not grow particularly well on nutrient agar, and so, in the latter part of the study, Difco tryptose blood agar base was substituted for the nutrient agar in this medium.

In view of the work of Brown (9) on the use of

horse blood for the differentiation of streptococci by hemolysis, and in anticipation that streptococci would be the principal organisms encountered, 5% defibrinated horse blood was used for making the blood agar plates in the North Okanagan study and in the first part of the general survey. As the work proceeded, however, appreciable numbers of staphylococci were encountered, and, since horse blood is unsuitable for the study of the hemolytic properties of staphylococci, it was decided to use the more readily accessible cow's blood for the blood agar plates. It was found that both streptococci and staphylococci grew well on this medium, and that hemolysis by both organisms could be readily distinguished. In order to conserve blood it was found necessary to reduce the concentration of blood from 5% to 3% for part of the study and the results obtained with this modification were entirely satisfactory.

Throughout the study, 0.005% of p-aminobenzoic acid was added to the basic medium for the blood agar plates in order to counteract the inhibitory action of sulfanilamide on any streptococci which might be present in the samples, in the event that the cow had been treated with sulfanilamide prior to sampling and that some of the drug had been shed in the milk.

The technique adopted for plating was as follows: 3-4 drops of the unincubated milk sample were used undiluted for each pour plate. Approximately 13 c.c. of medium were

employed to make each plate. The required amount of defibrinated blood was added to the melted and cooled basic medium just prior to pouring the plates. The plates were incubated at 37°C. for 24 hours. They were examined at the end of this time for type of growth, hemolysis, and relative numbers of the different types of colonies present. If there was sufficient growth, they were not incubated further. If, however, there was only slight growth in 1 day, the plates were incubated another 24 hours. This extended incubation period was usually found necessary for the plates made with Edwards' medium.

Owing to an insufficient supply of media in the North Okanagan study, only those samples were plated which appeared suspicious or positive by the other tests employed. Also, the lack of an incubator for this study, rendered the results from this test questionable, and so they are not included in the tables. The positive findings were, however, used as confirmatory evidence in classifying the quarters.

At first, identification of the organisms on the plates was accomplished by picking representative colonies into yeast litmus milk (Y.L.M.) and by examining smears made from the Y.L.M. cultures. Due to pressure of time, it was later found necessary to carry out the identification of the organisms by making gram stains directly from the colonies on the plates. It was found possible by this procedure to distinguish between chaining streptococci, staphylococci, spore-

forming and non-spore-forming rods and also between gram positive and gram negative bacteria. Time was not available for the further differentiation of the organisms.

Methods for Classifying the Organisms Associated With Mastitis.

During the course of the experimental herd study, it was found possible to examine the types of organisms present in the milk of all the lactating cows in herds #1 and #2. Representative colonies from the blood agar and Edwards' medium plates of all samples were picked into Difco tryptose phosphate broth containing 0.1% agar. Gram stains were made from the broth cultures, and on the basis of their morphology, the organisms were divided into three groups: streptococci, staphylococci and rod forms. In cases of doubt in differentiating between staphylococci and streptococci, the production of catalase was determined by placing a loopful of growth in a few drops of 3% hydrogen peroxide and observing for the formation of gas bubbles. Staphylococci produce catalase whereas the streptococci do not.

The organisms in each of the three groups were studied further, employing the methods devised for the classification of the particular group concerned. Before the various diagnostic tests were applied, however, the cultures were all replated on tryptose blood agar to ensure purity, and to determine the type of hemolysis. Pour plates were employed for the streptococci cultures and spread plates for the staphylococci and rod forms. The procedures used for the classification of the three groups of organisms are described below:

1. Streptococci.

The classification of the streptococci was carried out according to the methods of Plastridge et al (83) and Hansen (44). The tests employed were:

(a) the hydrolysis of sodium hippurate by the method of Coffey and Foley (25).

(b) the ability to split aesculin by a modification of the method of Diernhofer (29). The composition of the test medium used is as follows:

beef infusion:	500 c.c.
peptone:	5.0 g.
NaCl:	1.5 g.
K_2HPO_4:	1.0 g.
aesculin:	0.5 g.

The test, itself, was performed according to the procedure of Plastridge et al (83).

(c) final pH in 1% glucose broth after the method of Avery and Cullen (2), except that the basal medium used was nutrient broth and the pH of the medium was adjusted to 7.2. The pH reached at the end of 7 days incubation was determined with a Beckman pH meter.

(d) reaction in yeast litmus milk, using skim milk with the addition of 0.1% yeast extract and enough litmus solution to give a distinct pale violet colour. The cultures were examined at daily intervals up to 7 days for reduction, re-oxidation, acid production, type of clot and proteolysis.

(e) reduction of methylene blue milk. The test on the streptococci from herd #1 was carried out with a 1:5000

concentration of methylene blue (83). In the case of the organisms from herd #2, the concentration of methylene blue was reduced to 1:1000 (102).

(f) precipitin test. Brown's modification (10) of the Lancefield precipitin test was employed on a number of cultures using group A, B, and C sera.

(g) carbohydrate fermentation tests using casein peptic digest broth as the basal medium and 1.0% of the required carbohydrate. The medium was dispensed in 5 c.c. quantities and the various carbohydrates were added to the broth before sterilization. In the study on herd #1, the following sugars were employed: trehalose, sorbitol, salicin, raffinose and mannitol. In the study on herd #2, lactose was used in addition to the foregoing five carbohydrates. The cultures were incubated at 37°C. for 14 days and the amount of acid produced was determined by titration with N/4 sodium hydroxide using phenolphthalein as indicator.

2. Staphylococci.

The procedures of Plastridge et al (84) were employed to classify the staphylococci. They are as follows:

(a) reduction of nitrate according to the method of Plastridge et al (84) except that the concentration of potassium nitrate in the medium was reduced to 0.1%.

(b) liquefaction of nutrient gelatin, observations being made at intervals up to 14 days.

(c) reaction in yeast litmus milk as outlined above for the streptococci.

(d) pigment production on nutrient agar slants.

(e) utilization of ammonia nitrogen after the method of Plastridge et al (84) except that 0.0018% phenol red was used as indicator in the medium. This test was performed only on the cultures obtained from herd #2.

(f) coagulase test employing approximately 3-4 drops of a 1:2 dilution of human plasma in a sterile 3/8" by 2 7/8" (O.D.) corked test tube. The tubes were examined for coagulation after 1, 4 and 24 hours incubation at 37°C. This test, also, was performed only on the organisms isolated from herd #2.

(g) carbohydrate fermentation tests, using a nutrient broth base, with 1.0% of the required carbohydrate and 0.0018% phenol red as indicator. The sugars employed were lactose, sucrose, mannitol, raffinose and glycerol. Aqueous solutions of the various carbohydrates were prepared and sterilized by Seitz filtration. The basic broth, containing the indicator, was sterilized by autoclaving. After sterilization the broth and sugar solutions were mixed and dispensed aseptically into sterile tubes.

3. Rod Forms.

Unfortunately time did not permit the classification of the rod forms from herd #1. Those isolated from

herd #2 were studied in detail using the following procedures:

(a) reaction in yeast litmus milk as described above for the streptococci and staphylococci.

(b) reduction of nitrates as outlined for the staphylococci.

(c) liquefaction of gelatin as outlined for staphylococci.

(d) indol production from 1% tryptone broth.

(e) carbohydrate fermentation tests using the following sugars: lactose, fructose, galactose, mannitol, dextrin, glucose, glycerol, maltose and sucrose. The procedure used was identical with that used for the staphylococci except that 0.002% brom thymol blue was used in place of phenol red as the indicator.

Results and Discussion of Results.

The classification of the quarters as negative, suspicious or positive was based on the interpretation of the findings obtained by the use of the various tests applied to each quarter sample, employing the sum of the characteristics as a guiding principle. In general, the standards established for each test were those accepted by other workers in the field of mastitis. The results from all the quarters of each cow were considered as a unit, and all the available information was taken into consideration in classifying the respective quarters. It was thought that this procedure would give

a better picture of the mastitis condition of a given cow than would be the case if the standards of classification were rigidly set down.

The data are discussed under the general headings denoted at the beginning of the Experimental section. In the discussion of the results, the findings from each of the North Okanagan study, general survey, and experimental herd study have been considered separately. For the sake of convenience in the tables, the three projects have been designated by the following numbers:

I: North Okanagan study.
II: general survey.
III: experimental herd study.

The data from the three studies are recorded in every case in the above order.

1. Extent of Mastitis Infection in British Columbia.

The nine herds in the North Okanagan study together contained 104 cows. A number of these animals were tested more than once, giving a total of 121 cows studied and 484 samples tested. In the general survey, 435 different animals were examined, and these together with the number tested more than once gives a total of 510 animals and 1628 milk samples tested. There were 120 cows involved in the experimental herd study. Most of them were tested at least twice, giving a total of 279 animals and 1034 samples tested. Thus 3146 milk samples have been tested for mastitis during the course

of the entire study.

In compiling the data on the incidence of mastitis, the following procedure was adopted: in the case of cows tested twice, the results of each test were considered as 1/2, for those animals tested three times they were considered as 1/3, for animals tested more than three times the results were treated in a like manner. Thus the results from a single animal, regardless of the number of times tested, have the effect of a single test on the calculations. For the determination of the number of quarters infected in the general survey, all cows in which samples were taken from all four quarters were included, no adjustment being made for the number of times the animal was tested. This modification was found necessary because, in the case of a great many of the cows studied in the general survey, samples were submitted from only one or two quarters. In the North Okanagan study, two cows were in advanced stages of lactation, and so the results from these animals were not included in the calculations.

In Table 1 is found a summary of the findings on the extent of mastitis infection in the province as revealed by the present study. It can be seen that approximately 35% of the cows examined in the North Okanagan study and the experimental herd study were found positive for mastitis. This figure compares favorably with the results obtained by the other workers, whose findings have already been reviewed.

TABLE 1.

EXTENT OF MASTITIS INFECTION IN

BRITISH COLUMBIA.

Source of Data	Number of cows Studied	Percent of cows classified as			Number of Quarter Studied	Percent of quarters classified as		
		Negative	Suspicious	Positive		Negative	Suspicious	Positive
I	102	48.53	13.76	37.74	408	75.21	8.74	16.05
II	435	7.85	20.75	71.40	1432	27.37	30.52	42.11
III	120	38.50	27.48	34.02	468	59.08	23.81	17.11

A summary of the findings of these authors together with the results from projects I and III of the present study is contained in Table 2. These results indicate that mastitis infection in British Columbia is of approximately the same magnitude as in other parts of the world.

It is also observed from Tables 1 and 2 that, whereas approximately 35% of the cows studied were positive for mastitis, only about 17% of the total number of quarters examined were considered infected. These results are explained in Table 3 where it is seen that most of the positive cows studied were infected in one quarter only and that the percentage of cows infected in more than one quarter decreased inversely to the increase in the number of quarters involved. These findings are in keeping with the conception that chronic mastitis is a slow, progressive disease, which begins in one quarter of the udder and which, if not checked, gradually spreads to the other three quarters.

TABLE 3.

RELATIVE NUMBERS OF QUARTERS INFECTED IN INDIVIDUAL COWS.

Source of Data	Number of Positive Cows Studied	Percent of positive cows infected in			
		1 Quarter	2 Quarters	3 Quarters	4 Quarters
I	38.5	56.71	21.65	16.45	5.19
II	232	27.16	21.55	15.09	36.20
III	41	49.55	19.44	16.44	14.57

TABLE 2.

SUMMARY OF FINDINGS RELATIVE TO THE INCIDENCE OF MASTITIS IN VARIOUS PARTS OF THE WORLD.

Author	Locality	Number of Herds Studied	Number of Cows Studied	Percent of Cows Positive for Mastitis	Percent of Quarters Positive for Mastitis	Percent of Positive cows in individual Herds.
Rosell (87)	Quebec	--	1838	34.6	--	19 - 97
Bryan (11)	Michigan	322	2715	26.2	--	0 - 100
Gwatkin et al (42)	Ontario	28	594	39.0	26.4	15 - 91
Edwards (33)	England	18	809	36.4	16.4	10 - 84
North Okanagan Study	North Okanagan Valley	9	102	37.74	16.05	8 - 60
Experimental Herd Study	Lower Fraser Valley	4	120	34.02	17.11	10 - 42

It is seen from Table 2 that the incidence of mastitis in individual herds varies widely. This is probably due in part to the great variation in the size of the herds studied. It was found, for example, that the most seriously infected herd in the North Okanagan study consisted of only five cows and that three of these had mastitis. A similar number of infected animals in a larger herd would not have such a striking effect on the calculation of the percentage of infection in the herd. These data do, however, indicate that a great many herds are heavily infected with this disease.

The findings from the general survey, as recorded in Tables 1 and 2 are to be interpreted with caution. The samples studied in the course of this survey were taken, for the most part, from clinically positive cows and so do not constitute a representative sampling of the cow population. This accounts for the very high percentage of positive cows found in this study and also for the large number of infected quarters. Nevertheless, these results demonstrate that mastitis is a widespread and serious disease among the dairy cows of this province.

In the North Okanagan study, there were no active cases of mastitis at the time of sampling. The various owners, however, reported that a number of the cows studied had previously had attacks of the common chronic form of the disease. All forms of mastitis were encountered in the general survey - chronic, flare-up of chronic, sub-acute, acute,

and gangrenous. The history of the various cases at the time of sampling extended from a few hours in some instances to three years in others. The clinical history of the cows examined in the experimental herd study indicates that most of the mastitis in these herds was of a sub-clinical nature. Only four cases of acute flare-ups have been encountered in this study to date.

2. Factors Predisposing the Udder to Mastitis Infection.

During the course of the work reported upon herein, it was found possible to study a number of the factors which have been suspected as predisposing agents to udder infection. These are discussed below.

(a) Breed.

As can be seen from Table 4, there does not appear to be any relationship between the breed of the cow and the likelihood of its being infected with mastitis. The relative numbers of animals of the different breeds examined in the North Okanagan study were too few to permit the drawing of conclusions. The data from the other two studies, however, indicate that the four common breeds of dairy cattle are infected to approximately the same degree. In the general survey, the incidence of infection among the four breeds is seen to be of the order of 60-70%, whereas in the experimental herd study the level of infection is found to be approximately 30-40% for the four breeds. This difference in level

is due to the high proportion of positive cows included in the general survey. These findings are in exact agreement with the generally accepted idea that the incidence of mastitis is not influenced by the breed of the animal.

TABLE 4.

RELATION OF THE BREED OF COW TO THE INCIDENCE OF MASTITIS.

Source of Data		Jersey	Holstein	Guernsey	Ayrshire
I	No. of Cows Studied	60	13	5	8
	% Infected	44.17	23.08	00.00	37.50
II	No. of Cows Studied	139	125	47	24
	% Infected	68.35	72.80	59.58	75.00
III	No. of Cows Studied	56	22	42	--
	% Infected	34.82	40.91	29.33	--

(b) Age of Cow.

The results from the North Okanagan study and the experimental herd study (Table 5) demonstrate very clearly that the likelihood of a cow being infected with mastitis increases with increasing age of the animal. Again, the distribution of the cows studied in the general survey renders

the data from this study as unsuitable for determining the effect of the age of the cow on the incidence of mastitis, other than to show that even young animals are subject to considerable mastitis infection. In general, then, the results recorded herein agree with the findings of other workers that the chances of a cow being infected with mastitis increase as the number of lactations increases, and that very few cows of over eight years of age are completely free from evidence of infection (11).

TABLE 5.

RELATION OF THE AGE OF COW TO THE INCIDENCE OF MASTITIS.

Source of Data		2 - 4 Years	5 - 6 Years	7 - 9 Years	Over 9 Years
I	No. of Cows Studied	25	7	11	6
	% Infected	28.00	28.57	40.91	83.33
II	No. of Cows Studied	143	112	79	26
	% Infected	66.43	77.68	73.42	76.92
III	No. of Cows Studied	73	23	13	8
	% Infected	25.68	35.22	66.69	60.00

(c) Method of milking and sanitary conditions.

Owing to the lack of sufficient data relevant to the numbers of positive cows as compared to the total number of animals in individual herds, it is impossible to give any exact results on the effect of sanitary methods and method of milking on the incidence of mastitis in the herds studied. From the little data available, it would appear that these factors do not greatly influence the extent of mastitis infection in individual herds. It was observed that herds, in which the general sanitary practices were excellent, were as heavily infected with mastitis as those in which the sanitary conditions were poor. Some herds in which the animals were hand milked were seriously infected, whereas, some machine-milked herds were lightly infected.

(d) Trauma.

A number of the samples submitted during the general survey were obtained from clinical cases of mastitis, relative to which it was reported that the mastitis attack had been preceded by an injury to the udder or teats. These results indicate that injury is an important predisposing agent to udder infection. However, the small number of cases recorded does not permit the drawing of general conclusions.

In general, then, the findings from the study reported herein, agree with the accepted concepts that the breed of the animal and the method of milking have little

effect on the incidence of mastitis, but that advancing age and injury predispose to udder infection.

3. Evaluation of the Tests Used for the Detection of Mastitis.

In order to render the evaluation of the tests used for the detection of mastitis as reliable as possible, no samples were included in which factors other than mastitis may have contributed to a positive reaction. For this reason, the results from two cows, in the North Okanagan study, which were in an advanced stage of lactation, were omitted. Similarly, the data obtained in the general survey from cows which had freshened less than a week before the samples were taken, and from those which were almost dry at the time of sampling, were not used. Also, because the indirect tests used for the diagnosis of mastitis are not required to detect abnormalities in milk samples of grossly altered physical appearance, and because the reliability of the reactions of these tests is influenced by the presence of clots, serum, blood, etc., only those samples from the general survey which were normal in physical appearance were included for study on the tests for mastitis. For the same reason, nine samples from the experimental herd study which were grossly abnormal in physical appearance were withheld from the present study.

(a) Physical Examination of the Milk.

All of the milk samples examined in the North Okanagan study were of normal physical appearance. In the

experimental herd study, only 50 out of a total of 1034 samples tested were abnormal with respect to physical appearance. In most cases, the degree of alteration was very slight, and consisted merely of the presence of a few flakes or of a watery appearance. When these findings are considered in the light of the incidence of mastitis among the cows in these studies, it is readily observed that the predominant type of mastitis in these animals is of a sub-clinical nature, such that the physical appearance of the milk is not affected. It has also been found that it is only in the acute or advanced stages of the disease that the physical appearance of the milk is altered. Hence, it is obvious that tests other than the examination of the milk for physical abnormality must be resorted to, in order to detect the sub-clinical forms of mastitis infection. However, the presence of flakes or clots in the foremilk is often the first indication to the farmer of a flare-up of chronic mastitis, and so from this point of view, the physical examination of samples of foremilk is of considerable practical value.

In the general survey, the milk samples received for analysis presented a great variety of appearances - normal, flaky, clotty, serumy, etc. Some of the samples contained varying amounts of blood, others gave the appearance of coagulated serum, and still others were of a bright yellow colour. The secretion of such grossly abnormal milks, demonstrates the tremendous effect which advanced mastitis

infection has on the secretory cells of the udder.

(b) Modified Whiteside Test.

Murphy and Hanson (77), who developed the modified Whiteside test for mastitis, observed that its ability to detect udder infection paralleled closely that of the leucocyte count. Dunn et al (30) found that the Whiteside reaction was due to the presence of leucocytes in the milk, and that the intensity of the reaction increased with increasing leucocyte count. They postulated that the reaction is similar to that which takes place between nucleic acid and sodium hydroxide. They suggested, further, that the sodium hydroxide breaks down the leucocyte structure and forms a gelatinous mass with the cell nucleic acid, possibly with the formation of the sodium salt of the acid. It is thought that fat globules and serum solids are adsorbed on this gelatinous mass giving the appearance of white floccules.

The findings obtained with the modified Whiteside test in the present study are shown in Table 6. These data indicate considerable variation between the results of the three studies. If all reactions of ± or higher are taken to indicate udder disturbance, then, in the North Okanagan study, 92.44% of the negative samples and 96.20% of the positive samples would be correctly classified by this test. The results are, of course, not so definite with the suspicious samples. In the case of the general survey and the experimen-

tal herd study, however, there are found to be quite a number of false positive and false negative reactions.

TABLE 6.

THE USE OF THE MODIFIED WHITESIDE TEST IN THE DETECTION OF MASTITIS

SOURCE OF DATA	REACTION	PERCENT OF TOTAL NEGATIVE QUARTERS	PERCENT OF TOTAL SUSPICIOUS QUARTERS	PERCENT OF TOTAL POSITIVE QUARTERS
I	No. of Samples	357	40	79
	0	92.44	32.50	3.80
	±	6.16	17.50	11.39
	1+	1.40	32.50	17.72
	2+, 3+, 4+	--	17.50	67.09
II	No. of Samples	304	207	196
	0	61.18	44.45	10.71
	±	22.04	24.64	10.20
	1+	13.16	24.15	23.47
	2+, 3+, 4+	3.62	6.76	55.62
III	No. of Samples	558	276	191
	0	73.48	44.20	9.43
	±	16.13	22.10	13.09
	1+	9.50	25.00	41.36
	2+, 3+, 4+	0.89	8.70	36.12

The reason for this variability in the results from the three studies appears to be connected with the age of the

milk samples at the time of testing. In the North Okanagan study, the samples were examined for Whiteside reaction about 16-20 hours after being taken. In the experimental herd study, however, it was frequently found impossible to perform the Whiteside test until the milks were considerably over 20 hours old, the samples having stood at room temperature in the meantime. In the general survey the samples were often two or three days old on arrival at the laboratory. It was usually found that the reaction was less distinct with the older samples. This may have been due in part to the leucocytes having risen with the cream, and formed a dense mass which had protected the leucocytes from the action of the sodium hydroxide. In other cases, the concentration of the leucocytes in the cream layer may have resulted in a false positive reaction, if the sample had not been well shaken before the test was performed.

The results indicate that when the modified Whiteside test is applied to strictly fresh quarter samples of milk, it is a very accurate means of detecting the presence of abnormal conditions in the udder. Hence, the reliability of this test, together with the small amount of equipment required and the ease with which it can be performed, should render the modified Whiteside test of considerable value as a field test for mastitis.

(c) Geneva Blotter Test.

The Geneva blotter test is an adaptation of

the brom thymol blue tube test for the determination of the pH of milk, and was developed especially for use in the field as an aid in the detection of mastitis. The results obtained with this test are found in Table 7. These findings indicate that, while the test appears to have classified the negative samples fairly accurately, only 29.12% of the positive samples were correctly classified by this method. Also, 11.39% of the positive samples would have been considered negative had this test alone been employed to detect mastitis. It would appear, then, that the Geneva blotter test is not particularly sensitive to minor changes in the udder and that it is likely to classify a considerable number of positive quarters as free from infection. Possible explanations for these observations are discussed in the following section on the determination of pH using brom cresol purple.

TABLE 7.

THE USE OF THE GENEVA BLOTTER TEST

IN THE DETECTION OF MASTITIS

SOURCE OF DATA	REACTION*	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
I	No. of Samples	357	40	79
	N	93.84	45.00	11.39
	?	6.16	55.00	59.49
	G	---	---	29.12

*N: normal - yellow green colour.
?: suspicious - green colour.
G: garget (Positive) - blue green colour.

It should be emphasized that when the Geneva blotter test is used in the field, it should be employed only under well ventilated conditions, for any quantity of ammonia in the atmosphere from an accumulation of manure, will tend to give false positive reactions.

(d) pH Determination with Brom Cresol Purple.

It is generally found that, in cases of udder disturbance, the pH of the milk tends to become more alkaline than usual, the degree of the alkalinity depending on the severity of the upset. It is thought that, in mild cases of mastitis, this alkalinity is due to the increased permeability of the cell membranes to bicarbonates from the blood. In more advanced cases, it is likely that blood serum and possibly lymph fluids pass almost unchanged into the milk. Blood serum has a pH of about 7.4, whereas the pH of normal milk is about 6.4 - 6.8. Hence, it is reasonable to conclude that, in the milder forms of the disease where only a small part of the udder tissue is involved, not enough bicarbonate is secreted to have very much effect on the final pH of the milk. This would explain, them, why the pH test is not very sensitive to slight changes in the udder. The drastic changes which take place in cases of acute mastitis and advanced chronic mastitis where the udder metabolism is grossly affected, can readily be detected by this test.

The results obtained by the determination of

the pH of the milk samples, which are found in Table 8, substantiate the above statements and agree in general with the findings obtained with the Geneva blotter test, namely, that a great many positive samples are classified as negative by these tests. Hence this test is of value in differentiating the most seriously infected animals in a herd, but is not so reliable as the modified Whiteside test in detecting cases of mild or insipient mastitis.

The rather large proportion of negative samples in the North Okanagan study which appeared slightly alkaline to brom cresol purple, might indicate that the pH of the milk from normal cows in this area is slightly higher than that found elsewhere. The small number of samples studied, however, prevents the drawing of definite conclusions on this matter.

A few samples were encountered which were acid to brom cresol purple. These findings were always associated with very large numbers of streptococci, and it is thought that these organisms had formed enough acid in the milk to lower the pH sufficiently to change the colour of the brom cresol purple. Whether the milk was acid on leaving the udder or whether the change had been brought about during the interval between taking the samples and testing them is not known, but the latter explanation appears more tenable.

TABLE 8.

THE USE OF THE DETERMINATION OF pH WITH BROM CRESOL PURPLE IN THE DETECTION OF MASTITIS

SOURCE OF DATA	REACTION	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
I	No. of Samples	357	40	79
	N.	72.55	32.50	8.86
	V.Sl.Al.	2.24	---	---
	Sl.Al.	24.09	42.50	30.38
	Al.,V.Al.,V.Sl. Ac.,Sl.Ac.,Ac.	1.12	25.00	60.76
II	No. of Samples	304	207	196
	N.	68.75	39.13	18.37
	V.Sl.Al.	13.16	19.81	12.76
	Sl.Al.	9.21	23.19	19.39
	Al.,V.Al.,V.Sl. Ac.,Sl.Ac.,Ac.	8.88	17.87	49.48
III	No. of Samples	557	272	188
	N.	71.14	27.21	11.17
	V.Sl.Al.	17.32	17.28	11.17
	Sl.Al.	10.24	27.21	25.00
	Al.,V.Al.,V.Sl. Ac.,Sl.Ac.,Ac.	1.30	28.30	52.66

TABLE 8 Cont'd.

KEY TO REACTIONS.

N.: normal (pH approx. 6.4 - 6.8)
V. Sl. Al.: very slightly alkaline (pH approx. 6.9)
Sl. Al.: slightly alkaline (pH approx. 7.0 - 7.1)
Al.: alkaline (pH approx. 7.2 - 7.3)
V. Al.: very alkaline (pH approx. 7.4 or higher)
V. Sl. Ac.: very slightly acid (pH approx. 6.3)
Sl. Ac.: slightly acid (pH approx. 6.2 - 6.1)
Ac.: acid (pH approx. 6.0 - 5.6)

It should be emphasized at this point that colostrum has an alkaline pH, and that the milk from animals late in their lactation period also tends to show a high pH. The extent of this rise in pH during late lactation varies with the individual cow, some animals showing a much greater change than others. Hence, the pH test is of no value in the detection of mastitis in animals in either early or late lactation. Also, since the alteration in the pH of the milk in insipient cases of mastitis is so slight, the test should be used only on quarter samples of milk, as a mild infection in one quarter would not be detected if the pooled milk of all four quarters of the udder were examined. Another factor to be considered in this connection is the report by Kleckner (61) that the pH of milk tends to rise slightly during estrum. The possible effect of this rise in pH on the validity of the pH test for the detection of mastitis has not as yet received attention. The problem, however, is worthy of study.

During the course of this study, it was observed that

the different shades of colour associated with the various pH's of the milk could be more readily distinguished with brom cresol purple than with brom thymol blue as was used in the Geneva blotter test. The former indicator, then, is suggested as being more suitable in determining the pH of milk as an aid in detecting mastitis.

(e) Hotis Test.

The Hotis test as originally proposed by Hotis and Miller (49), was claimed to be 95% accurate in detecting the presence of Str. agalactiae in milk samples, as compared to the blood agar plate method. Miller (70) later reported that Staph. aureus could also be detected by this test. Str. agalactiae forms canary yellow flakes at the bottom and along the sides of the tubes, whereas Staph. aureus tends to form white flakes with rust-coloured margins. Miller stated further that other mastitic organisms do not produce such flakes in incubated milk samples. He believes, however, that this does not detract from the value of the test, since Str. agalactiae is the only organism of importance in a control program for the eradication of mastitis, and that if this organism can be accurately detected then the test has served its purpose.

The results obtained from the Hotis test in the present study are shown in Table 9. It will be noted that all of the samples examined in the general survey were

used in the preparation of this table. As can readily be seen, very little information is revealed by these figures. There are several reasons for this. The large percentage of positive samples which were graded negative by this test may have been due to the fact that for most of the cases studied, the causative organism was probably not Str. agalactiae nor Staph. aureus. It is thought, also, that in the general survey, the udder staphylococci may have outgrown the Str. agalactiae present, when the samples were held at fairly low temperatures during transit, such that the Str. agalactiae were unable to form their characteristic flakes when the milks were subsequently incubated at 37°C.

In a great many instances, doubtful reactions were encountered in which there was usually a slight amount of yellow sediment in the tubes even after 48 hours incubation. It was difficult to determine whether such reactions were positive tests for Str. agalactiae, or whether they merely represented the beginning of the formation of a clot in the tube. Unfortunately, the organisms isolated on the blood agar plates from the milk samples could not be identified, and so the number of false positive and false negative reactions of the Hotis test are not known. Bryan and Devereux (14) and Berry and Clark (6) also found difficulty in interpreting suspicious reactions obtained with this test. Bryan and Devereux, in fact, observed many false positive reactions which they found to be caused by udder micrococci. These workers also found that the

results of the Hotis test were not constant on repeated testing of the milk from negative and positive cows. In contrast to these findings, consistent results were obtainted with the microscopic and blood agar plate methods when applied to the same milk samples.

TABLE 9.

THE USE OF THE HOTIS TEST IN THE DETECTION OF MASTITIS DUE TO STREPTOCOCCUS AGALACTIAE

SOURCE OF DATA	REACTION	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
II	No. of Samples	381	369	606
	Negative	90.03	81.57	84.32
	Pos. for Str. agalactiae	7.09	13.28	13.86
	Pos. for Staph. aureus	2.88	5.15	1.82
III	No. of Samples	557	272	188
	Negative	70.56	50.37	55.32
	Pos. for Str. agalactiae	8.62	32.72	29.25
	Pos. for Staph. aureus	20.82	16.91	15.43

McCulloch and Fuller (67) determined that the Hotis reaction was due to an agglutination phenomenon, and that the agglutinating factor was present in the milk of most cows even

though the milk was negative to the Hotis test. They found also, that streptococci other than Str. agalactiae occasionally gave positive reactions with this test.

The results obtained in the present study, together with those reported by the above authors, indicate that the Hotis test is not of very great value in the detection of mastitis. This test may be useful among animals badly infected with Str. agalactiae, but where organisms other than Str. agalactiae are found to any extent to be responsible for mastitis, the test is of little value. Also, the number of false positive reactions which are frequently obtained, render its use questionable. Hence, in view of the fact that almost as much information can be obtained by a microscopic examination of incubated milk samples, the use of the Hotis test for the detection of mastitis is not warranted.

(f) Determination of Percent Chlorine.

The results obtained by the determination of the percent chlorine in the milk samples is shown in Table 10. Various workers disagree as to what percent chlorine should be taken as the dividing point between normal and mastitic samples. Rosell (87), whose method was followed in the work reported herein, stated that a chloride content in the milk of over 0.142% should be considered an indication of mastitis infection. Blood and Rowlands (7) on the other hand, from a study of several methods for the determination of the

chlorine content of milk, concluded that Rosell's method gave results approximately 25% higher than methods in which the protein is precipitated or otherwise removed before the chlorine titration is made. Despite these disagreements, however, it should be possible to set a standard for each method employed. The results reported in the present paper reveal, that, whereas almost all of the positive samples had a chlorine content of over 0.142%, an appreciable number of negative samples had a chlorine content between 0.142% and 0.160%, and a few negative samples even had a chlorine content of over 0.160%. These findings may indicate that 0.160% is a better figure than 0.142% to be taken as the dividing line between negative and positive samples with this procedure. They may also indicate that the chlorine content of the milk from normal cows in the areas from which these samples were obtained is higher than in the localities where Rosell made his studies.

Davies (28) found that Ayrshire milk was slightly higher in chlorine content than Shorthorn and Guernsey milk. Sharp and Struble (100) observed that milk from Holstein cows had a higher chlorine content than that from Jerseys and Guernseys. They attributed this, in part, to the fact that there are more interfering proteins in Holstein milk which might affect the end-point of the titration such that higher readings might result. These workers also found that the chlorine content of colostrum is high, and that the chlorine content of the milk drops rapidly during the first few days after calving,

reaches a minimum, slowly rises during the first 60% of the lactation period, then increases more rapidly, and finally rises very rapidly during the last 10% of the lactation period.

It is thought that the chlorine content of milk changes as occasion demands in order to maintain isotonic conditions between the milk and the secretory cell protoplasm. If for any reason the solids-not-fat content of the milk should be decreased, the chlorine content rises accordingly. It is known that the solids-not-fat content of milk is affected by such factors as type of feed, drought, etc., and that the chlorine content of the milk is thus indirectly affected by the same factors. It has been found that one molecule of sodium chloride is isotonic with two molecules of lactose and it is thought that the high chloride content of mastitic milk may be due to a disturbance in the synthesis of lactose, so that the amount of lactose secreted is reduced and hence the chlorine content of the milk is increased.

In the present study, the value of the chlorine test in the detection of mastitis was found to be greatly reduced in the case of late lactation animals. A considerable number of samples, from cows late in lactation, which appeared normal by the pH test and leucocyte count were decidedly abnormal with respect to percent chlorine.

TABLE 10.

THE USE OF THE CHLORIDE TEST

IN THE DETECTION OF MASTITIS

SOURCE OF DATA	CHLORINE CONTENT OF MILK IN PERCENT	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
I	No. of Samples	153	18	49
	<0.124	12.42	---	2.04
	0.124-0.142	41.83	5.56	---
	0.142-0.160	30.72	16.66	2.04
	0.160-0.178	9.15	27.77	14.29
	>0.178	5.88	50.01	81.63
II	No. of Samples	304	207	195
	<0.124	20.40	7.24	1.03
	0.124-0.142	39.80	10.63	3.59
	0.142-0.160	22.37	20.29	7.69
	0.160-0.178	9.54	22.71	13.33
	>0.178	7.89	39.13	74.36
III	No. of Samples	492	270	188
	<0.124	31.50	8.52	3.19
	0.124-0.142	35.98	14.07	1.59
	0.142-0.160	25.81	16.67	3.19
	0.160-0.178	5.69	19.63	16.49
	>0.178	1.02	41.11	75.54

It was also observed that the chlorine content of milk drops rapidly during the milking process. In a preliminary experiment with 8 cows, it was found that, whereas in a number of cases samples of foremilk were positive for mastitis as judged by the percent chlorine and pH determinations, samples taken after the milking machine had been removed, appeared normal. The greater the initial reading, the greater appeared to be the reduction as milking proceeded. It is thus very evident that when use is made of the chloride and pH tests for the detection of mastitis, samples of foremilk must be used. The removal of two or three streams of milk before the samples are taken in order to eliminate contaminating bacteria should not alter the results from these tests to any extent. It appears that the other tests employed in the detection of mastitis are not so greatly affected by the use of first or last strippings as the chloride and pH tests. No data is available from the present study regarding the effect of the breed of the animal on the chloride content of normal milk.

It may be concluded, then, that the chloride test is too sensitive to minor changes in the udder cell metabolism to be of value as a field test in mastitis control work. This conclusion was also arrived at by Frayer (40). In view of the fact that the chloride test is so readily affected by the stage of the lactation period, and considering that cows vary greatly in the length of their lactation periods, it is

likely that this test, if used as the sole means of detecting mastitis infection in the udder, may report considerable numbers of false positive reactions.

(g) Leucocyte Count.

The findings obtained with the leucocyte count are shown in Table 11. These results indicate that most of the negative samples had a leucocyte count below 500,000 per c.c., but they reveal, further, that appreciable numbers of the positive samples also had a leucocyte count below 500,000. These findings, then, suggest that this test is of about the same value in detecting evidence of mastitis as the pH test, in that both of these tests tend to give a considerable number of false negative reactions. However, the fact that only a 2 mm. loopful of milk was spread over a 1 sq. cm. area in place of the 0.01 c.c. employed under Standard Methods, may account for some of these irregular results obtained with the leucocyte count.

It should be remembered in interpreting the results of the leucocyte count, that the number of leucocytes in colostrum is very high, and that the leucocyte count of the milk of normal animals tends to increase in late lactation.

It is probable that if a leucocyte count of over 100,000 rather than one of over 500,000, as is usually accepted, be taken to indicate a mastitic condition, less positive samples would be graded as negative by this test. Also,

if the leucocyte count is combined with the examination of the smear for the types of organisms present, the use of the two tests should be of considerable value in the detection of abnormal conditions in the udder and of the organisms responsible for such disturbances.

TABLE 11.

THE USE OF THE LEUCOCYTE COUNT

IN THE DETECTION OF MASTITIS

SOURCE OF DATA	LEUCOCYTE COUNT IN THOUSANDS PER c.c. OF MILK	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
I	No. of Samples	357	40	79
	<100	80.11	27.50	13.93
	100-500	19.33	52.50	22.78
	500-1,000	0.56	15.00	13.92
	>1,000	---	5.00	49.37
II	No. of Samples	301	203	191
	<100	75.08	44.83	6.80
	100-500	21.93	38.42	9.43
	500-1,000	1.99	12.81	24.09
	>1,000	1.00	3.94	59.68
III	No. of Samples	517	254	176
	<100	85.69	65.36	13.63
	100-500	13.73	20.87	24.43
	500-1,000	0.58	12.20	22.16
	>1,000	---	1.57	39.78

(h) Microscopic Study of the Organisms Present in the Milk Samples.

A number of interesting facts are revealed by the microscopic examination of the smears made from the milk samples, the results of which are found in Table 12. It can be readily seen that a considerable number of positive samples showed no organisms at all on the smear. In the case of the experimental herd study, this is especially noteworthy, since all of the milks had been incubated for 16 to 18 hours before the smears were made. It would thus appear that, in many cases of obvious udder disturbance, no detectable number of organisms were being shed in the milk. Whether "non-specific" mastitis was present in such instances, or whether it so happened that the causative organism was not being shed in the milk at the time the samples were taken, is not known.

It can also be seen that a greater proportion of the positive samples harboured streptococci than did the negative samples. The reverse relationship was found to exist for staphylococci and rod forms. Hence, it would appear that, in the diseased udders, the mastitic streptococci had replaced the normal udder micrococci. On comparing the results of this test with those obtained with the blood agar plate technique, the findings from which are shown in Table 13, it can be seen that a greater number of samples harbouring streptococci was revealed by the latter test.

TABLE 12.

THE USE OF THE MICROSCOPIC EXAMINATION OF MILK SAMPLES IN THE DETECTION OF MASTITIS

SOURCE OF DATA	PREDOMINANT TYPES OF ORGANISMS IN EACH SAMPLE	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
II	No. of Samples	383	376	623
	No Organisms	42.30	46.28	47.35
	Chaining Streptococci, alone & with Staph. & Rods	15.40	19.15	30.34
	Staphylococci alone & with Rods	24.28	25.26	15.25
	Rods & Diplococci	18.02	9.31	7.06
III	No. of Samples	558	276	191
	No Organisms	31.18	22.10	27.75
	Chaining Streptococci, alone & with Staph. & Rods	3.23	25.00	39.79
	Staphylococci alone & with Rods	37.45	32.97	21.99
	Rods & Diplococci	28.14	19.93	10.47

The microscopic examination of the smears made from the milk samples appears to be of considerable value in the rapid detection of the types of organisms responsible for cases of mastitis. The test requires less time and fewer materials than the blood agar plate method, and, if applied to incubated samples of milk, should reveal the great majority of

the types of organisms present in the milk samples.

It should be noted at this point that, in compiling the data for both the microscopic and cultural studies of the milk samples for Tables 12 and 13, the results from all of the samples studied in the general survey were employed.

(i) Cultural Study of the Organisms Present in the Milk Samples.

The results obtained from the cultural study of the types of organisms present in the milk samples using blood agar plates are found in Table 13. The data obtained by the use of this test are similar to those found with the microscopic examination of the milk samples. A few positive samples were again found which did not harbour any definite organisms. Also the proportion of streptococci was again observed to be much greater in the positive than in the negative samples. However, the percentage of streptococci found in the positive samples by this method was considerably higher than that found by the microscopic method. It is of special interest to note the difference in the proportion of non-hemolytic staphylococci to hemolytic staphylococci in the negative and positive samples. In the negative samples the two types of staphylococci were present in about equal numbers. In the positive samples, on the other hand, there were approximately three times as many hemolytic as non-hemolytic types. These findings would suggest, then, that hemolytic staphylococci are more definitely associated with mastitis than are the non-hemolytic types.

TABLE 13.

THE USE OF BLOOD AGAR PLATES IN THE DETECTION OF ORGANISMS ASSOCIATED WITH MASTITIS

SOURCE OF DATA	PREDOMINANT TYPES OF ORGANISMS IN EACH SAMPLE	PERCENT OF TOTAL NEGATIVE SAMPLES	PERCENT OF TOTAL SUSPICIOUS SAMPLES	PERCENT OF TOTAL POSITIVE SAMPLES
II	No. of Samples	383	376	623
	No Significant Growth	6.00	4.52	1.92
	N.H. Staph. alone & with Rods	43.34	25.80	11.08
	H. Staph. alone & with N.H. Staph. & Rods	31.86	32.71	35.31
	Streps. alone & with Staph. & Rods	6.00	28.20	45.59
	Rods	12.80	8.77	6.10
III	No. of Samples	558	276	191
	No Significant Growth	16.31	7.61	6.81
	N.H. Staph. alone & with Rods	27.06	17.39	9.42
	H. Staph. alone & with N.H. Staph. & Rods	36.38	23.91	26.18
	Streps. alone or with Staph. & Rods	7.88	40.22	50.26
	Rods	12.37	10.87	7.33

N.H. Staph: non-hemolytic staphylococci.
H. Staph: hemolytic staphylococci.
Streps: streptococci (both non-hemolytic and hemolytic)

It is generally agreed that the hemolytic properties of the various streptococci associated with mastitis bear no relationship to the ability of the organisms to produce udder infection. In fact, different strains of Str. agalactiae have been found to exhibit alpha, beta, weak beta and gamma haemolysis as described by Brown (9). With the staphylococci, however, the non-hemolytic types are considered to be non-pathogenic, and the hemolytic types, especially if this property is associated with the power to coagulate human or rabbit plasma, are thought to be probable pathogens. The hemolytic staphylococci encountered in the present study usually formed clear zones around the colonies on blood agar. Although hemolytic staphylococci can be isolated from the healthy udder, the finding of considerable numbers of these organisms, combined with other indications of disease, may be considered evidence that the causative organism in such cases is the staphylococcus isolated.

Regarding the use of Edwards' medium as a rapid means of detecting streptococci in milk samples, it was observed in the present study that very rarely were streptococci found on the Edwards' medium plates and not on duplicate plates made with ordinary blood agar. Admittedly, the differentiation of Str. uberis, by the property of this organism to form dark areas around the colonies on Edwards' medium, cannot be accomplished by the use of ordinary blood agar. Otherwise, since Edwards' medium reveals only streptococci, and since it

has been found in the present study that organisms other than streptococci are likely to be present in a fairly large proportion of the milk samples, it appears that tryptose blood agar is a satisfactory general plating medium for routine studies on the types of organisms associated with mastitis.

It may be concluded that the blood agar plate technique is useful in the detection of the types of organisms present in the milk from animals suspected of having mastitis. This technique is required for the isolation of organisms from the milk samples in order to differentiate in greater detail between the various types of mastitic organisms. This detailed study is necessary in order to determine whether a case of streptococcic mastitis is of the common contagious form due to Str. agalactiae or whether it is of the less contagious types caused by Str. dysgalactiae and Str. uberis. The use of blood agar plates is also of value in differentiating between hemolytic and non-hemolytic staphylococci, although some indication of whether the staphylococci present in a milk sample are normal udder bacteria or are probable pathogens may be obtained by noting the relative numbers of staphylococci observed in different milk samples on the microscopic examination of the milks. It is probable that, if the predominant types of organisms responsible for the mastitis in a given herd, were once determined by cultural methods, then, on subsequent testings of this herd, the blood agar plate method need not be employed.

4. Detailed Study of the Organisms Associated with Mastitis.

The cultures obtained by picking representative colonies from the blood agar plates of the milk samples from all of the cows in herds #1 and #2 of the experimental herd study, were subjected to a variety of tests, which were selected to detect the organisms most commonly associated with mastitis.

In Table 14 are found the differentiating characteristics employed to distinguish between the species of streptococci which have been found capable of producing mastitis infection. The information contained in this table has been compiled from the data of Hansen (44), Sherman (102), Merchant and Packer (68) and Plastridge et al (83).

On the basis of the findings of Merchant and Packer (68) and Plastridge et al (83) that none of the pathogenic streptococci ferment raffinose, whereas many of the saprophytic types do ferment this carbohydrate, all of the raffinose-fermenting strains isolated were considered as being saprophytic, and were not classified further. Over half of the streptococcus cultures obtained were found to be Str. agalactiae, Str. dysgalactiae or Str. uberis. Some of the strains isolated were found to be atypical with respect to one or two properties. These organisms were classified as belonging to the species they most resembled. Str. zooepidemicus and Str. pyogenes were not encountered. There were a num-

TABLE 14.

REACTIONS USED TO DIFFERENTIATE BETWEEN MASTITIS STREPTOCOCCI

Organism	Hippurate Hydrolysis	Aesculin Splitting	Hemolysis	Lancefield Group	Carbohydrate Fermentation					Final pH in Glucose Broth	Reduction of Methylene Blue Milk	Reaction in Litmus Milk		
					Trehalose	Sorbitol	Salicin	Mannitol	Raffinose			Reduction Before Clotting	Reduction After Clotting	Days to Clot
Str. agalactiae	+	0	variable.	B	+	0	+	0	0	4.4-4.6	0	0	+	1-3
Str. dysgalactiae	0	0	variable.	C	+	0	0	0	0	4.7-4.9	0	+	+	1--
Str. uberis	+	+	variable.	?	+	+	+	+	0			0	+	2--
Str. zooepidemicus	0	+	β	C	0	+	+	0	0	4.6-4.9	0			
Str. pyogenes	0	+ -	β	A	+	0	+	0	0	5.0-5.2	0			

ber of strains which did not fit into any of the recognized species; these cultures have been designated as "unclassified".

For the rapid differentiation of the common mastitic streptococci, Brown's modification of the Lancefield precipitin test (10), together with the determination of the ability of the organisms to hydrolyze sodium hippurate and split aesculin appear to be satisfactory test procedures. If more precise work is desired, the reaction in milk, the final pH in glucose broth and the fermentation of trehalose, sorbitol, salicin and mannitol may be employed.

In the classification of the staphylococci, the descriptions of the properties of Staph. aureus given by Bergey (5) and Minett (72) were employed as a guide. The other staphylococci encountered were differentiated according to the system proposed by Hucker (50). About one third of the staphylococcus cultures studied were Staph. aureus. Another quarter of the cultures could not be fitted into any of the recognized species. The other staphylococci encountered appeared to be of the types usually found in the normal udder. The following species were found in the milks of both herds: Micrococcus albus, M. candidus, and M. epidermidis. M. caseolyticus, M. citreus, M. flavus, and M. luteus were also isolated from the samples from herd #2.

In evaluating the methods used to classify the staphylococci, it would appear from the present study that Staph. aureus can be readily identified by its ability to hemolyze

cow's blood agar, coagulate human blood plasma, ferment mannitol, reduce nitrate to nitrite and liquify gelatin. It seems evident, however, that the methods employed to differentiate the udder micrococci should be revised. In Hucker's system of classification, the primary divisions are determined on the basis of the type of pigment produced by the various organisms. Since it is well known that a given species may show considerable variation in the type of pigment formed, depending on environmental conditions, it is obvious that such a system of classification is not very sound.

In any attempt to revise the classification of these organisms, a study would first have to be made using recognized species of micrococci. It is probable that such properties as gelatin liquefaction, nitrate reduction, action on milk and fermentation of carbohydrates could be utilized in such a scheme. Another complicating factor in the classification of these organisms is that a number of workers, including Bergey (5), divide the staphylococci from the micrococci merely on the basis of the size of the individual bacterial cells, and the way in which they form groups. It has been found in the present study that such morphological characteristics vary greatly depending on the medium the organism is grown in. There is also the question as to whether the micrococci and staphylococci should be considered as separate genera or whether these organisms, which have many characteristics in common, should be grouped under one genus.

It may be concluded, then, that the classification of the staphylococci and micrococci is far from satisfactory, and that considerable work on this group of organisms is urgently needed.

The rod forms isolated from the various samples were found difficult to classify. Most of them appeared to be types which are widely distributed in nature, and which are frequently found in the milk from normal animals, but which, because of their inactivity in various test media, have been studied very little. They were classified as far as was possible by the descriptions given in Bergey (5). The rods isolated from the milks of herd #1 were not very extensively studied, but it was determined that approximately half were species of corynebacteria, the rest being other gram positive types and gram negative bacteria. The rod forms isolated from herd #2 were studied in greater detail. About half of these were found to be of the following types: Microbacterium flavum, Microb. liquefaciens, Microb. lacticum, C. flavidum, C. bovis (Bacterium lipolyticum), and C. xerose. Approximately one third of the rods isolated from herd #2 were gram positive forms which apparently do not fit into any of the groups described by Bergey. A few gram negative types were encountered: Proteus sp., Serratia sp., and Ecoli, but it appears likely that these organisms were contaminants or chance inhabitants of the udder, and were not related in any way to mastitis.

Because most of these rod forms appear so inactive, it is probable that they do not play a very significant role in the initiation of mastitis infection in the udder. The only rods which commonly produce mastitis are E. coli, Aer. aerogenes and C. pyogenes, and these forms can be readily identified. It is not known whether or not the other rods, which may be considered normal inhabitants of the udder, play a part in predisposing the udder to infection by other invading bacteria. It may be noted in this connection that the majority of the Corynebacterium and Microbacterium types were isolated from negative quarters in which no other types of bacteria were found. It would appear, from the evidence at hand, that the rods commonly incriminated in mastitis can be fairly easily identified by recognized procedures, and that, in a large-scale mastitis program, it is unnecessary to study in detail the rod forms isolated from apparently healthy quarters or from positive quarters in which pathogenic staphylococci and streptococci are present.

A total of 424 cultures were classified for herd #1 and 523 for herd #2. Because of the fact that, in a great many cases, it was found that surface and sub-surface colonies of the same organism had been picked from the original plates, it was decided to study the general findings on the classification of the organisms from the point of view of the predominant types of bacteria found in each quarter, taking into consideration the probability of the organisms present being

potential producers of mastitis. These general results are found in Table 15.

TABLE 15.

PREDOMINANT TYPES OF ORGANISMS IN QUARTER MILK SAMPLES FROM ALL OF THE ANIMALS IN HERDS #1 AND #2

HERD	TYPES OF ORGANISMS	PERCENT OF TOTAL NEGATIVE QUARTERS	PERCENT OF TOTAL SUSPICIOUS QUARTERS	PERCENT OF TOTAL POSITIVE QUARTERS
#1	No. of Quarters	47	41	19
	No Organisms	10.64	4.88	---
	Saprophytic Staph, Streps, Rods	57.44	56.10	10.53
	Staph. aureus	29.79	12.19	5.26
	Str. agalactiae	---	17.07	52.63
	Str. dysgalactiae	---	4.88	5.26
	Str. uberis	---	2.44	5.26
	Unclassified Str.	2.13	---	---
	Combinations of Streptococci	---	2.44	10.53
	Combinations of Streptococci with Staph. aureus	---	---	10.53
#2	No. of Quarters	74	34	20
	No Organisms	6.76	---	---
	Saprophytic Staph, Streps, Rods	33.78	20.59	5.00
	Staph. aureus	35.14	32.35	30.00
	Str. agalactiae	1.35	5.88	25.00
	Str. dysgalactiae	8.11	2.94	10.00
	Str. uberis	1.35	---	---
	Unclassified Str.	4.05	2.94	---
	Combinations of Streptococci	---	11.77	5.00
	Combinations of Streptococci with Staph. aureus	9.46	23.53	25.00

In considering the findings for herd #1, it is seen that the predominant organisms in the negative quarters were saprophytic types and Staph. aureus. In the positive quarters, however, the most commonly found organism was Str. agalactiae. It would thus appear likely that the mastitis in this herd was due almost entirely to Str. agalactiae.

In herd #2, on the other hand, Staph. aureus, Str. agalactiae, and combinations of Staph. aureus and streptococci are seen to be present in about equal numbers in the positive quarters. It would seem probable, then, that a number of infective agents were present in this herd, a fact which might render difficult an attempt to control the disease in this herd by a management program.

The high incidence of Staph. aureus in the samples studied is noteworthy. Chapman et al (23), in a study of staphylococci isolated from human sources, concluded that any staphylococcus which was hemolytic on cow's blood agar, which coagulated human plasma and which fermented mannitol should be considered as a possible pathogen. Plastridge et al (84) came to the same conclusion regarding staphylococci isolated from mastitic animals, and stated that in the routine examination of milk samples, any staphylococcus which produced hemolysis on cow's blood agar or which was associated with over 500,000 leucocytes per c.c. should be considered as probably pathogenic. The significance which is to be attached to the isolation of a large proportion of Staph. aureus from otherwise

normal quarters is difficult to determine. No conclusions can be drawn from the study of only two herds, but it is probable that this organism may be more commonly distributed in normal udders than is generally recognized.

5. Studies on the Control of Mastitis.

Various workers have demonstrated that entire herds can be freed from Str. agalactiae infection, and can be maintained in such a condition for considerable periods of time, by a rigid management, segregation and treatment program. Among these workers are Plastridge et al (83) and Minett et al (74). Such a program involves the segregation of infected animals from healthy ones, the elimination from the herd of the animals considered unlikely to respond to treatment, the treatment of animals harbouring Str. agalactiae, a rigid control of the sanitary practices employed during the milking procedure to prevent the spread of the organism, and general measures to eliminate factors which might predispose to infection of the udder.

Hucker and Harrison (52) found in their studies that while they could not maintain a herd free from animals discharging mastitic streptococci in their milk, they could maintain a herd free from animals secreting abnormal milk. It should be emphasized, however, that their studies were confined to three herds only.

Watts (108) states that, if the incidence of Str. agalactiae infection in a herd is below 15%, the management

practices are not responsible for the amount of infection in the herd, but that, if the incidence is over this figure, the system of management is contributing to the incidence of the disease in the herd, and that if the management is rigidly controlled, a decrease in the amount of infection should result.

In the experimental herd study reported herein, it had been hoped to demonstrate that the findings of Plastridge et al (83) and Minett et al (74), that Str. agalactiae could be eliminated completely from a herd, could be duplicated in this area. The results from the first three routine testings of herds #1 and #2 and from the first two testings of herds #3 and #4 are shown in Table 16. It is observed that in all except herd #3 there was no significant reduction in the amount of infection as the study proceeded. In order to explain some of these findings, a brief discussion on each of the four herds is given below.

Herd #1 was found to be highly infected with Str. agalactiae, although only three clinical cases of mastitis were encountered during the course of the study. It would appear that this herd should respond to a management program for the elimination of Str. agalactiae. The facts that no treatment measures had been employed in this herd during the period of the work reported herein, and that none of the clinical cases had been eliminated from the herd, may explain why the incidence of mastitis in this herd has not decreased. It is evident, however, that the management program has at least pre-

vented the disease from spreading to a greater extent throughout the herd.

TABLE 16.

PERCENTAGE INFECTION IN THE EXPERIMENTAL HERDS AT THE TIME OF THE VARIOUS ROUTINE TESTINGS

		PERCENT OF COWS IN HERD			
		HERD #1	HERD #2	HERD #3	HERD #4
FIRST TEST	No. of Cows	28	35	14	23
	Neg.	42.86	54.29	35.71	43.48
	Susp.	28.57	11.42	42.86	17.39
	Pos.	28.57	34.29	21.43	39.13
SECOND TEST	No. of Cows	27	33	10	27
	Neg.	33.34	15.16	40.00	48.15
	Susp.	33.33	42.42	50.00	14.81
	Pos.	33.33	42.42	10.00	37.04
THIRD TEST	No. of Cows	23	33		
	Neg.	34.78	27.28		
	Susp.	34.78	36.36		
	Pos.	30.44	36.36		

As has been shown in the previous section on the classification of the organisms associated with mastitis, herd #2 was found to harbour a variety of mastitic organisms - Str. agalactiae, Str. dysgalactiae, Str. uberis, and Staph. aureus. Several cases of clinical mastitis were recorded during the

period of study. The presence in this herd of a number of organisms in addition to Str. agalactiae probably explains why the herd has not responded to the control program.

Herd #3, which is a small and carefully supervised herd, has shown a considerable lessening in the incidence of mastitis during the course of the study. None of the common mastitic organisms could be isolated from the milk samples from this herd at either of the routine testings. A number of pseudomonas-type rods were observed to be present in the milks from most animals in the herd, but no relationship could be established between the amount of infection in the herd and the distribution of these organisms.

The predominant organisms in herd #4 were found to be staphylococci. The absence of Str. agalactiae probably accounts for the fact that little success has been attained with the control program in this herd.

It should be re-emphasized that any control program is of value only if the predominant organism in the herd is Str. agalactiae. Such a condition was found in only one of the experimental herds. Complete success, therefore, cannot be expected in the other three herds, although some lessening of the incidence of the disease may be anticipated in these herds, because of the fact that the adoption of the rigid management practices, outlined in the schedule to the agreement and referred to previously in this report, should eliminate most of the factors which might predispose the udder to infection by

other mastitic organisms.

6. Studies on the Treatments Used for Mastitis.

Ever since mastitis has become a serious problem to the dairy farmer, various investigators have worked towards the development of suitable treatment measures for this disease. Because of the variety of organisms which have been found to be capable of causing mastitis, the problem of prescribing suitable treatment measures becomes very complicated. It is, of course, necessary to determine whether the case at hand is of the infectious or non-infectious type of mastitis, since treatment with chemotherapeutic or antibacterial agents is of little value in curing non-infectious mastitis. It is also useful to know the causative organism in a given case of mastitis, because specific agents are often best suited for eliminating certain bacteria, and may have little effect on other organisms.

Mixed bacterial vaccines and autogenous vaccines were among the first treatment measures tried. Their use, however, met with irregular success. In some herds the vaccines appeared to have assisted in the lessening of the incidence of the disease, but the results were not conclusive enough to prove definitely that these methods were wholly responsible for the elimination of mastitis infection. It appears that the common mastitis streptococci and staphylococci do not induce the production of a very high level of antibodies in the blood.

Numerous chemotherapeutic and several antibiotic agents have been employed from time to time in the treatment of mastitis. The most widely used of these are discussed briefly below.

One of the first of these chemotherapeutic agents to be used in the treatment of chronic mastitis was colloidal silver oxide which is commonly known as Novoxil. Very favourable reports have been made regarding the effectiveness of udder infusions of this product in the removal of mastitic organisms from the udder. However, the fact that this preparation is very toxic to udder tissue, and frequently produces a mild induration in the udder and teats, precludes it from extensive use. This compound is definitely contraindicated in cases of acute mastitis.

Certain of the acridine dyes, namely acriflavin (trypaflavin) and proflavin, have also been used extensively in the treatment of mastitis. Schalm (93) reported favourable results from infusing the udder with trypoflavin in hypertonic (20%) sugar solution in eliminating Str. agalactiae. Johnson (58) was successful in treating dry and almost dry cows harbouring Str. agalactiae with acriflavine.

Schalm (92) reports having used Entozon in the treatment of mastitis due to Str. agalactiae by udder infusion methods. This preparation has the following composition:

2, 3-dimethoxy-6-nitro-9-(y-diethyl-amino-β-oxy-propylamino) acridene dihydro-chloride 5.88%

2-ethoxy-6, 9-diamino-acridine lactate	29.44%
amyl saccarine	58.80%
sodium biborate	5.88%

The treatment was successful in most cases with very little resulting irritation to the udder. However, the milk from the treated cows could not be used for one to three weeks after treatment, and the presence of milk or other exudate was found to precipitate the acridine derivatives in the preparation, necessitating the frequent injection of the diseased quarter with the drug.

Bryan et al (15) reported the successful treatment of sub-clinical streptococcic mastitis with infusion into the affected quarter of 75 c.c. quantities of a 1:1000 aqueous solution of phemerol (para-tertiary-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate). The use of this product, however, tends to induce the production of flaky milk for several days, and the mucous membranes lining the teat and milk cisterns become temporarily thickened. Because of these facts, it is recommended that this treatment be applied near the end of the lactation period.

The sulfa drugs, especially sulfanilamide and sulfathiazole, have been extensively used in the treatment of mastitis, the former compound being more effective against streptococci and the latter against staphylococci. These drugs are best administered as udder infusions, and are very suitable for injection into dry udders, the preparation being left in the udder until the cow freshens. It is often recommended in

acute cases that sulfanilamide be administered orally until the acute symptoms in the udder have subsided.

Favourable results were obtained by Klein et al (62) in the treatment of Str. agalactiae and staphylococcic infection with sulfanilamide. Swett et al (105) found sulfanilamide and sulfadiazine alone and in combination to be effective in curing mastitis due to streptococci, staphylococci and coliform organisms. These drugs, however, were only about 50% effective in eliminating Ps. aeruginosa infection.

Tyrothricin and its two components, tyrocidin and gramicidin, the products of the organism Bacillus brevis, have also been used with considerable success in the treatment of mastitis. These products are highly toxic to the udder tissue, and care must be taken in determining the dosage to be used, and in deciding how long the preparations are to be left in the udder. Little (65) found tyrothricin and gramicidin to be effective in curing chronic streptococcic mastitis.

Bean et al (3) recorded favourable results in treating chronic streptococcic mastitis in lactating cows with the following products arranged in order of decreasing efficiency: acriflavin, Novoxil, Entozon, and tyrothricin. In dry cows, tyrothricin was found to be most effective in eliminating streptococci, with Entozon, acriflavin, and Novoxil following in order of decreasing effectiveness.

Schalm (94) reported on the use of Entozon, trypaflavin, neutral acriflavin, tyrothricin and Novoxil in the treatment

of chronic streptococcic mastitis, and found Entozon, tyrothricin and Novoxil to be of most value for this purpose. He found staphylococcic infection relatively resistant to chemotherapy. Schalm later (95) concluded that, in order to reduce to a minimum the amount of milk lost during the treatment of lactating animals, only clinical cases should be treated during lactation with tyrothricin or sulfanilamide in oil, and that these cases should be treated only enough to relieve the clinical symptoms. The organisms should be completely eliminated from the udder by the treatment of the dry cow with such products as silver oxide.

The 1942 report of the Chief of the United States Bureau of Animal Industry (18) records findings similar to those of Bean et al and Schalm. It was found that acriflavin, colloidal silver oxide, Entozon, and tyrothricin, in order of decreasing efficiency, to be effective in curing lactating cows, and tyrothricin, Entozon, acriflavin and silver oxide, in order of decreasing effectiveness, to be of value in treating dry cows.

Penicillin, the antibiotic agent formed by Penicillium notatum, is being very widely used at the present time in the treatment of mastitis. This substance is active against gram positive organisms but apparently has very little effect on gram negative bacteria. It is very suitable for administration by udder infusion, because it is relatively non-toxic and dissolves readily in water and saline.

Foley et al (38) and Seeley et al (99) have carried out in vitro studies on the effect of penicillin on the organisms commonly associated with mastitis, and have found that the following organisms were susceptible to small amounts of the drug: Str. agalactiae, Str. uberis, Str. dysgalactiae, Str. zooepidemicus, Staph. aureus and C. pyogenes. E. coli was not destroyed by the preparation.

Schalm (91) found staphylococcic mastitis more resistant to penicillin therapy than the streptococcic form of the disease. He also found that a greater proportion of dry udders responded to penicillin treatment than did lactating udders. He recommends an initial treatment of 5 doses to be administered at 5 successive milkings. If the organism is still present 10-14 days after treatment, he suggests repeating the treatment with 4 injections 12-24 hours apart using double the original dosage.

The 1945 report of the Chief of the United States Bureau of Animal Industry (19) records variable success with the administration of one treatment only of 50,000 units of penicillin, and strongly suggests that penicillin should not be considered a panacea for mastitis, but that prevention is still the best treatment for this disease.

Riddet (31) suggests that the use of a bacteriophage may be effective in the treatment of mastitis due to staphylococci. He isolated several phages against mastitic staphylococci, and found that the phage preparations were non-toxic

to the udder tissue. He did not report, however, how successful the phage preparations were in eliminating the organisms from the udder.

Washburn (107) concluded from his studies that if cows are fasted for a five day period once every lactation period, there is a definite tendency towards the elimination of mastitis. As a result of this procedure, he found the pH and percent chlorine of the milk of mastitic animals to return to normal, and for flakes and clots in the milk to disappear. He did not report, however, how well the mastitic organisms were eliminated by this treatment.

Pounden (85) recommends the cessation of milking in acute attacks of mastitis due to Str. agalactiae until the inflammatory symptoms have subsided. He found that the cessation of milking, for up to three days, had no ill effects on the animal, and that the causative organisms were effectively eliminated by this procedure. Such a treatment, however, was considered to be of no value in cases of mastitis due to staphylococci and C. pyogenes.

Despite all the progress which has been made in the treatment of mastitis, the best treatment for this disease is prevention. A certain treatment may eliminate the mastitic organisms from an udder, but it does not protect that udder from becoming reinfected in the future with the same or a different organism. It has usually been found that cows which

have had attacks of mastitis and have been successfully cured are more susceptible to infection than healthy animals (58). Hence, care in the prevention of the spread of mastitis infection from already diseased to healthy cows in the herd, and vigilance in the elimination of factors which might predispose the udder to infection, are the best treatments which can be recommended for this disease.

It should also be emphasized that the greater the extent of induration and fibrosis in the udder, the less likely is the treatment applied to be successful. It is thus apparent that the best results will be obtained by treatment in the early stages of the disease. Further, the fact that the infection of the udder with streptococci is thought to begin at the base of the teat and to work upwards in the udder, whereas staphylococcic and coliform infections take place in the parenchymatous tissue itself, may help to explain why mastitis due to streptococci is usually more responsive to treatment measures than that caused by staphylococci and coliform organisms.

It is generally agreed that the most effective method of administering the various therapeutic agents used for the treatment of mastitis is by infusion directly into the udder. Oral or intravenous methods appear to be of little value, with the possible exception of the oral administration of sulfanilamide in acute cases of the disease. The dosage and number of treatments to be prescribed vary, depending on the size

of the udder, the infecting organism, the severity of the attack, the extent of the udder tissue involved, and the length of time the disease has been present in the udder.

In the general survey reported herein, a number of therapeutic agents were used by the practising veterinarians in the treatment of mastitis. The most widely used treatment measure was the infusion of the udder with a mixture in mineral oil of sulfanilamide and sulfathiazole. Unfortunately, no data could be collected on the effectiveness of the various treatments applied.

In the experimental herd study, an experiment using six cows in herd #2 was performed, in order to study the effectiveness of penicillin in the treatment of streptococcic mastitis. The animals selected for study were all seriously infected with streptococcic mastitis. 5 animals were given an initial treatment of 25,000 units of penicillin per quarter, and the sixth was given 37,500 units per quarter. 4 of the first 5 animals were given a second treatment of 25,000 units per quarter 24 hours after the first. 2 of these 4 cows were given a third treatment of 25,000 units per quarter 24 hours after the second. Milk samples were obtained from all 6 animals one week after the last treatment. The results of the examination of the milk samples showed that there was no apparent reduction in the numbers of streptococci in the infected quarters as a result of the treatment. This may have been because too little penicillin was administered, or because the treatments

were not given frequently enough, or because the cows treated were too severely infected with mastitis to respond to treatment. These results indicate that there is scope for considerable work on the treatment of mastitis in the herds under study by the Research Council.

GENERAL DISCUSSION.

The results of the studies reported herein indicate that the extent of mastitis infection in the dairy herds of British Columbia is of the same order as that reported for other parts of the world. This disease, then, is so widespread and produces such serious consequences as to create a definite problem for the dairy industry.

Of the many tests which have been employed for the detection of mastitis infection, none appears to give a complete picture of the case at hand. The use of a large number of tests yields considerable information regarding the status of a particular infection. Yet, it is probable that a single or a few tests applied very frequently, e.g. at weekly or half-weekly intervals, would give more information regarding the changes taking place in the udder, than would a large number of tests applied at less frequent intervals. The finding of abrupt changes or trends towards more positive reactions with one or two tests would be of greater value in the study of a case of chronic mastitis than would be the finding of a suspiciously positive reaction with a number of tests on a single

occasion. The value of such a procedure is demonstrated in the work of Johns and Hastings (55), who found that there was a tendency in normal cows for the biochemical reactions of the milk to be slightly higher at the afternoon milking, and for the total bacterial count to be higher at the morning milking. They thus recommended the examination of the milk of an animal from several consecutive milkings as being necessary for the proper diagnosis of mastitis infection.

Some tests, however, appear to be of greater value than others for the detection of mastitic milk. The Hotis test is useful only where there is a high incidence of mastitis due to Str. agalactiae. Definite positive findings are probably 100% accurate in the diagnosis of this type of mastitis, but negative findings do not necessarily signify freedom from disease. In view of the fact that it should be possible to observe the presence of streptococci on the microscopic examination of incubated milk samples, the Hotis test is found to be non-essential as a diagnostic test for mastitis.

The value of the leucocyte count depends on the obtaining of a uniform smear. When this determination, properly carried out, is combined with the examination of the smear for the presence of mastitic organisms, considerable information is obtained regarding the extent of inflammation in the udder, and the organisms producing such disturbances.

Of the tests which might be applied to field use, as in a control program, the determination of the pH of the milk

appears to be unreliable. This test undoubtedly reveals the most seriously infected cows in a herd, but it fails to detect a large number of samples which should be positive as revealed by other tests. The determination of the chlorine content of the milk, on the other hand, appears to be too sensitive to slight changes in the composition of the milk to be of value as a field test.

On the basis of the results obtained in the present study, the modified Whiteside test is recommended for use in the field. Although some difficulty has been encountered in the interpretation of some of the findings of this test as a result of the different ages of the samples tested, the use of this test at frequent intervals on strictly fresh samples of milk is to be recommended as an aid in detecting abnormalities in the udder. This test could be readily carried out in the barn with very little equipment by a non-technical person.

A physical examination of the udder is a useful adjuct to studies on the milk from a suspicious animal. Such an examination gives an indication of the extent of the udder which has been affected by the disease, and so would aid in determining whether or not it would be advisable to apply treatment to such an udder.

The present state of our knowledge of mastitis indicates that mastitis caused by Str. agalactiae is the only form of the disease which spreads extensively through an entire herd. Str. agalactiae is the only mastitic organism, with the possible exceptions of Str. dysgalactiae and Str. uberis, which

lives almost exclusively in the udder and its secretion. The other organisms capable of producing mastitis infection are constantly present in the environment of the animal and special conditions appear to operate when these organisms produce disease. Because of their ubiquitous nature, it would be impossible to try to eliminate these organisms from a herd.

It has been demonstrated by a number of workers that Str. agalactiae can be eliminated from a herd, and that the herd can be maintained in this condition for considerable periods of time. In order to accomplish this objective, however, it is necessary to maintain a rigid management program in the herd with special attention to milking procedures, and to combine this program with the segregation of infected animals and the prompt treatment of animals found to harbour Str. agalactiae.

The average farmer does not appreciate the contagious nature of common chronic mastitis, and he considers the program necessary for its eradication to be beyond practical application. However, there is no other known way to control the disease, since the successful treatment of an animal following an attack of mastitis does not prevent reinfection of the udder at a later date.

In the case of mastitis due to organisms other than Str. agalactiae, a rigid control program as described above, appears to be of little value in controlling the incidence of the disease in a herd, except insofar as the sanitary practices

employed aid in the maintenance of more healthful conditions in the herd.

In herds #3 and #4 in the experimental herd study reported herein, the infective agents were found not to be Str. agalactiae, and it would appear that the probability of completely eradicating the disease from these herds by a control program only is very slight. The finding of a variety of streptococci in the milk samples from herd #2 may help to explain why this herd has not responded as favourably as might have been expected to the management program. Herd #1 is apparently suitable for a management program study, since the dominant organism in the herd is Str. agalactiae.

An important problem in the study of mastitis is the question of what constitutes infection. If the term infection is taken to mean the invasion of the tissues of the host by pathogenic agents, and the production of pathological changes in such tissues, does the mere finding of potential mastitic organisms in the milk of a cow, in the absence of other evidence of abnormality, indicate that the cow is infected? Is the organism merely existing in the udder in a commensal state under such conditions? What factors are responsible for its multiplication or its disappearance from the udder? Should it be necessary to treat apparently normal cows found to be shedding Str. agalactiae in their milk?

The fact that mastitic organisms can be isolated with great regularity from the milk of apparently healthy udders,

suggests that a study of the relationships between the organism and the host is urgently required in order to understand fully the mastitis problem. Is it that chronic mastitis, because of its slow development, at first affects the quantity of milk secreted and not so much the composition of the milk, except in cases of a flare-up? Why do apparently harmless udder bacteria on occasion produce severe reactions in the udder? Do these organisms, under some conditions, acquire increased virulence? Do certain apparently normal udder staphylococci predispose the udder to infection by mastitic streptococci?

Can the limited bacterial flora of the normal udder be explained by the selective action of the bactericidal substances of the udder? Are these substances reduced in quantity or altered in composition when disease is produced in the udder? Why, during the course of an attack of mastitis, is it sometimes impossible to isolate any organisms, and yet there is definite evidence of inflammation as determined by chemical tests and leucocyte count of the milk? Why, at other times, in the same case, are the chemical reactions of the milk normal, but a very high count of organisms are shed in the milk?

The bacteriology of the flora of the milk from mastitic animals has been pursued to the extreme from a practical point of view. It has been found that mastitis due to Str. agalactiae can be controlled by a rigid management program, and that the organisms commonly causing mastitis are susceptible to various

therapeutic agents which may be used to treat cases of mastitis. It is also known that mastitis due to organisms other than Str. agalactiae cannot be completely held in check by a control program. However, very little is known about what actually takes place in the udder when the organism is establishing itself prior to the production of visible evidence of disease. It is the writer's opinion that the explanation for many of the problems associated with the development of mastitis infection in the udder would be answered if a concerted effort were made to determine some of the inter-relating factors between pathogen and host. This information could best be obtained by the intensive study of a few infected animals under controlled conditions, observations on the chemical and bacterial contents of the milk being made at daily or twice daily intervals over a period of time. It appears evident to the author that, through such a study, a significant contribution would be made to our knowledge of mastitis and of the means to combat this disease.

Based on the findings of this study and on those reported by other workers, it is clear that a large-scale control program for the lessening of mastitis infection in the dairy herds of British Columbia should be initiated without delay. One of the first problems in such a program would be the education of the farmer, in order to make him realize that the rigid management practices recommended are absolutely essential to keep the disease in check in his herd. Besides fol-

lowing sanitary milking procedures and methods for the elimination as far as possible of factors which might predispose the udder to infection, the farmer should be encouraged to maintain the size of his milking herd with heifers raised on the farm or by the purchase of maiden or in-calf heifers. The purchase of mature cows should be discouraged. It is also evident that some action should be taken to suppress the purchase of mastitic animals by dealers, and the subsequent sale of these animals to farmers at auction sales.

To carry on such a program, a number of trained field men would be required to supervise the work on the farm, to perform physical examinations of the udders of the cows, and to carry out field tests on the milk from all animals in the herds. It is recommended that the initial study of a herd should consist of a physical examination of the udders of all cows in the herd, together with a microscopic examination of incubated quarter samples of milk for numbers of leucocytes and types of organisms present. If it appears necessary in certain cases, blood agar plates could be made to distinguish between non-hemolytic and hemolytic staphylococci and to isolate streptococci. If it seems advisable to differentiate between Str. agalactiae and other mastitic streptococci, the colonies on the plates can be picked, and Brown's precipitin test (10) applied without much difficulty. After this initial test, it proposed that the herds be examined regularly by the modified Whiteside test, which could be performed at about

monthly intervals by the field man at the farm.

It is the writer's opinion that further preliminary experimental work on the general problem of mastitis as applied to British Columbia is not warranted, that the time is suitable for the initiation of a widespread control program in this province, and that the methods outlined above for its execution are adequate and easily performed.

SUMMARY AND CONCLUSIONS.

The significant findings from two years' work by the B.C. Industrial and Scientific Research Council on the problem of mastitis in the dairy herds of British Columbia have been reviewed.

It has been found that the incidence of mastitis in British Columbia is comparable to that reported for other parts of the world.

It has also been determined that the breed of the cow has no effect on the incidence of udder infection, that injury is a predisposing factor to mastitis, and that the age of the cow is related to infection, in that the older the cow, the more likelihood of its being infected.

Of the various tests employed for the detection of mastitis, the pH determination appeared least sensitive and the chlorine determination most sensitive to changes in the udder. The Hotis test was found unsuitable for general use where the incidence of organisms other than Str. agalactiae is high.

The leucocyte count and blood agar plate technique were considered efficient but time consuming. The modified Whiteside test shows promise as a field test for the rapid detection of diseased conditions in the udder.

The results obtained with respect to the control of mastitis were not extensive enough to denote progress. The fact that, in three of the four herds studied in this program, the dominant organisms were found not to be Str. agalactiae, may help to account for the apparently negative results obtained.

As a result of these studies it is concluded that a study should be made of the inter-relationships between the invading organism and the host in the period during which the organism is establishing itself in the udder.

It is also concluded that a large-scale control program should be initiated at once in this province in order to check the spread of the disease and to provide for its eventual eradication. An outline of a suggested program is given in the General Discussion.

BIBLIOGRAPHY

1. --- Better milking procedure and mastitis control. Canad. Dairy and Ice Cream J., 24, 43-44 & 86, 1945.

2. AVERY, O.T. & CULLEN, G.E. The use of the final hydrogen ion concentration in differentiation of Streptococcus haemolyticus of human and bovine types. J. Exp. Med., 29, 215-234, 1919.

3. BEAN, C.W., MILLER, W.T. & HEISHMAN, J.O. Chemotherapy of streptococcal bovine mastitis. Am. J. Vet. Res. 4, 344-352, 1943.

4. BEAN, C.W., MILLER, W.T. & HEISHMAN, J.O. A note on Corynebacterium pyogenes as the cause of bovine mastitis. J. Am. Vet. Med. Ass'n, 103, 200-202, 1943.

5. BERGEY, D.H., BREED, R.S., MURRAY, E.G.D., & HITCHINS, A.P. Bergey's Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore, 5th Ed., 1030 pp., 1938.

6. BERRY, J.C., & CLARK, F.C. Mastitis in the dairy cow as revealed by field and laboratory tests. M.S.A. Thesis, University of B.C., 89 pp., 1937.

7. BLOOD, J.W. & ROWLANDS, A. The chlorine content of milk as an indication of mastitis. J. Dairy Res., 7, 47-54, 1936.

8. BROADHURST, J., CAMERON, G. & MacLEAN, M.E. A filterable virus isolated from mastitis cows. Cornell Vet., 29, 261-270, 1939.

9. BROWN, J.H. The use of blood agar for the study of streptococci. Monograph of the Rockefeller Institute for Med. Res., No. 9, 1919.

10. BROWN, J.H. A simplified method for grouping hemolytic streptococci by the precipitin reaction. J. Am. Med. Ass'n, 111, 310-311, 1938.

11. BRYAN, C.S. The incidence of streptococcic mastitis among dairy cattle. Vet. Med., 32, 70-74, 1937.

12. BRYAN, C.S. Penicillin and mastitis control. Milk Plant Monthly, 35, 27-28 & 65, 1946.

13. BRYAN, C.S. & BRYAN, P.S. The viability of certain udder infection bacteria in butter made from raw cream. J. Milk Tech., 7, 65-67, 1944.

14. BRYAN, C.S. & DEVEREAUX, E.D. A comparison of the microscopic test, Hotis test and blood agar plate in detecting streptococci of mastitis in milk. Cornell Vet., 27, 68-74, 1937.

15. BRYAN, C.S., HEDEMAN, L.P., VISGER, E.E., & CUNKELMAN, J.W. Phemerol in the treatment of 176 cows with chronic streptococcic mastitis. Vet. Med., 39, 417-420, 1944.

16. BULL, L.B., MUNCH-PETERSEN, E., MURNANE, D. & MacLEAN, J.D. Studies on bovine mastitis. 1. Study of an experimental herd. Div. of Anim. Hlth. and Nut., Coun. for Sci. & Ind. Res., Commonwlth. of Aust., Bul. No. 134, 107 pp., 1940.

17. --- Report of the Chief of the Bur. of Anim. Ind., Agric. Res. Admin., U.S.D.A., 1941, p. 10.

18. --- Report of the Chief of the Bur. of Anim. Ind., Agric. Res. Admin., U.S.D.A., 1942, p. 4.

19. --- Report of the Chief of the Bur. of Anim. Ind., Agric. Res. Admin., U.S.D.A., 1945, 27-28.

20. --- Report of the Chief of the Bur. of Dairy Ind., Agric. Res. Admin., U.S.D.A., 1943, p. 16.

21. BURKHARDT, S., BEACH, B.A. & SPENCER, G.R. Aerobacter aerogenes associated with acute toxemic mastitis in eleven cows. J. Am. Vet. Med. Ass'n, 103, 381-383, 1943.

22. --- The Canada Year Book, 1945, Dom. Dep't Stat., Dep't Trade & Comm., 211-214 & 217-226.

23. CHAPMAN, G.H., BERENS, C., PETERS, A. & CURCIO, L. Coagulase and hemolysin tests as measures of the pathogenicity of staphylococci. J. Bact., 28, 343-363, 1934.

24. CLARK, F.C. The production of undesirable odours in raw milk by specific organisms associated with udder infections. B.S.A. Thesis, University of B.C., 21 pp., 1936.

25. COFFEY, J.M. & FOLEY, G.E. An improved medium for the demonstration of hydrolysis of sodium hippurate by streptococci. Am. J. Pub. Hlth., 27, 972-974, 1937.

26. CONE, J.F. Pseudomonas aeruginosa in bovine mastitis. J. Agric. Res., 58, 141-147, 1939.

27. CRABTREE, J.A. & LITTERER, W. Outbreak of milk poisoning due to a toxin-producing staphylococcus found in the udders of two cows. Am. J. Pub. Hlth., 24, 1116-1122, 1934.

28. DAVIES, W.L. The chloride content of milk. J. Dairy Res., 9, 327-335, 1938.

29. DIERNHOFER, K. Aesculinbouillon als Hilfsmittel für die Differenzierung von Euter-und Milchstreptokokken bei Massenuntersuchungen. Milch. Forsch., 13, 368-374, 1932.

30. DUNN, H.O., MURPHY, J.M. & GARRETT, O.F. Nature of the material in milk responsible for the modified Whiteside test for mastitis. J. Dairy Sci., 26, 295-303, 1943.

31. --- Editorial. Dairy Ind., 10, p. 323, 1935.

32. EDWARDS, S.J. Studies on bovine mastitis. IX. A selective medium for the diagnosis of streptococcic mastitis. J. Comp. Path. & Ther., 46, 211-217, 1933.

33. EDWARDS, S.J. Studies on bovine mastitis. X. The value of field and laboratory tests for the diagnosis of chronic streptococcic mastitis. J. Comp. Path. & Ther., 47, 49-60, 1934.

34. EVANS, A.E. The bacteriology of milk freshly drawn from normal udders. J. Inf. Dis., 18, 437-476, 1916.

35. FERGUSON, J. The bacteriology of acute mastitis. Cornell Vet., 30, 299-309, 1940.

36. FERGUSON, J. A bacteriological study of the infections which follow injury to the bovine udder. Am. J. Vet. Res., 5, 87-92, 1944.

37. FINCHER, M.G. Recent developments in the control of mastitis. Milk Plant Monthly, 34, 40 & 82, 1945.

38. FOLEY, E.J., LEE, S.W. & EPSTEIN, J.A. The effect of penicillin on staphylococci and streptococci commonly associated with bovine mastitis. J. Milk Tech., 8, 129-133, 1945.

39. FRANCIS, J. A bacteriological examination of bovine tonsils and vaginas. The possible relationship of the findings to mastitis and pneumonia. Vet. J., 97, 243-251, 1941.

40. FRAYER, J.M. Mastitis laboratory tests and their interpretation. J. Milk Tech., 7, 89-97, 1944.

41. GORINI, C. Mammary microflora in relation to cheese-making. Int. Rev. of the Sci. & Pract. of Agric., N.S. 3, No. 1, 22 pp., 1925.

42. GWATKIN, R., HADWEN, S., & Le GARD, H.M. Bovine mastitis: Notes on incidence, aetiology and diagnosis. Canad. J. Comp. Med., 1, 7-16, 1937.

43. --- Handbook on Mastitis. Practical suggestionsthat may help to prevent the spread of mastitis within the herd. Comm. on Public., Mastitis Res. Proj., B.C. Ind. and Sci. Res. Coun., Public. No. 1, 12 pp., 1944.

44. HANSEN, P.A. The identity of Streptococcus agalactiae. N.Y. Agric. Exp. Sta. Tech. Bul. No. 232, 44 pp., 1935.

45. HARRISON, J. A note on the examination of nose, throat & vaginal swabs and of dung samples for the presence of Sc. agalactiae. Vet. Rec., 54, p. 51, 1942.

46. HASTINGS, E.G. & BEACH, B.A. The production of milk of abnormal composition by animals free from udder streptococci. J. Agric. Res., 54, 199-220, 1937.

47. HOERLEIN, A.B. Private communication.

48. HOLFORD, F.D. Mastitis and its effect upon the milk industry. Cornell Vet., 20, 190- , 1930, via (42).

49. HOTIS, R.P. & MILLER, W.T. A simple test for detecting mastitis streptococci in milk. U.S.D.A. Circ. No. 400, 6 pp., 1936.

50. HUCKER, G.J. Studies on the Coccaceae. IX. Further studies on the classification of the micrococci. N.Y. Agric. Exp. Sta. Tech. Bul. No. 135, 31 pp., 1928.

51. HUCKER, G.J. Mastitis. V. The presence of mastitis streptococci in bovine mammary tissue. N.Y. Agric. Exp. Sta. Tech. Bul. No. 241, 21 pp., 1937.

52. HUCKER, G.J. & HARRISON, E.S. Mastitis. IX. The maintenance of a herd free from mastitis. N.Y. Agric. Exp. Sta. Tech. Bul. No. 246, 25 pp., 1937.

53. HUCKER, G.J., REED, M.S. & SAVAGE, E.S. Mastitis. VII. The relation of bovine mastitis to milk production. N.Y. Agric. Exp. Sta. Tech. Bul. No. 244, 97 pp., 1937.

54. JOHNS, C.K. & GIBSON, C.A. The influence of "abnormal" milk upon the yield and quality of cheddar cheese. J. Dairy Res., 13, 287-294, 1944.

55. JOHNS, C.K. & HASTINGS, E.G. Concerning the use of indirect biochemical tests for the diagnosis of chronic contagious mastitis. Canad. J. Res., 16, Sect. D, 6-14, 1938.

56. JOHNS, C.K. & HASTINGS, E.G. The relation between bacterial numbers and biochemical values in milk from streptococcus-free quarters. Canad. J. Res., 16, Sect. D, 15-30, 1938.

57. JOHNS, C.K., HICKS, T.J. & GIBSON, C.A. The influence of "mastitis" upon the yield and quality of cheddar cheese. J. Dairy Res., 11, 298-304, 1940.

58. JOHNSON, S.D. Observations on the treatment of mastitis with acriflavine. Cornell Vet., 31, 127-148, 1941.

59. JOHNSTON, T. Anatomical and experimental study of the teat of the cow with particular reference to streptococcal mastitis. J. Comp. Path. & Ther., 51, 69-77, 1938.

60. JONES, F.S. & LITTLE, R.B. The bactericidal property of cow's milk. J. Exp. Med., 45, 319-335, 1927.

61. KLECKNER, A.L. Some observations on the H-ion concentration of cow's milk during estrum. J. Am. Vet. Med. Ass'n, 46, 316-318, 1940.

62. KLEIN, L.A., KLECKNER, A.L. & PARRY, R.M. Reaction of the cow to sulphanilamide and its effect on mastitis streptococci and staphylococci. Vet. Ext. Bul., Univ. Penn., 39, No. 75, 3-21, 1939.

63. LITTLE, R.B. Bovine mastitis. I. The significance of the dose factor in the production of experimental mastitis. Cornell Vet., 27, 297-308, 1937.

64. LITTLE, R.B. Bovine mastitis. II. The production of mastitis by the suction of streptococci into the duct of the teat. Cornell Vet., 27, 309-316, 1937.

65. LITTLE, R.B. Gramicidin and tyrothricin therapy in chronic streptococcic mastitis. Milk Plant Monthly, 33, 31-34, 1944.

66. LITTLE, R.B. & FOLEY, E.J. Staphylococci associated with mastitis. J. Am. Vet. Med. Ass'n, 87, (N.S. 40), 637-649, 1935.

67. McCULLOCH, E.C. & FULLER, S.A. A study of streptococci producing positive Hotis reactions. J. Bact., 38, 447-459, 1939.

68. MERCHANT, I.A. & PACKER, R.A. Handbook for the etiology, diagnosis and control of infectious bovine mastitis. Burgess Pub. Co., Minneapolis, Minn., 66 pp., 1944.

69. MILLER, W.T. Bovine mastitis. N. Am. Vet., 17, 32-41, 1936.

70. MILLER, W.T. The Hotis test for the detection of mastitis bacteria in milk. U.S.D.A. Circ., No. 672, 7 pp., 1943.

71. MINETT, F.C. Bovine mastitis. A short review of present knowledge. J. Dairy Res., 2, 84-90, 1930.

72. MINETT, F.C. Studies on bovine mastitis. XII. Mastitis due to staphylococci. J. Comp. Path. & Ther., 50, 101-121, 1937.

73. MINETT, F.C. & MARTIN, W.J. Influence of mastitis and of Brucella abortus upon the milk yield of cows. J. Dairy Res., 7, 122-144, 1936.

74. MINETT, F.C., STABLEFORTH, A.W. & EDWARDS, S.J. Studies on bovine mastitis. VIII. The control of chronic streptococcus mastitis. J. Comp. Path. & Ther., 46, 131-138, 1933.

75. MUNCH-PETERSEN, E. Bovine mastitis. Survey of the literature to the end of 1935. Imp. Bur. of Anim. Hlth., Rev. Ser. No. 1, 272 pp.

76. MURPHY, J.M. The relationship of teat mucous membrane topography to age, breed, and incidence of udder infection in cows. Cornell Vet., 35, 41-47, 1945.

77. MURPHY, J.M. & HANSON, J.J. A modified Whiteside test for the detection of chronic bovine mastitis. Cornell Vet., 31, 47-55, 1941.

78. MURPHY, J.M. & HANSON, J.J. Infection of the bovine udder with coliform organisms. Cornell Vet., 33, 61-77, 1943. Via Dairy Sci. Abstr., 5, 1943, p. 92.

79. MURPHY, J.M., PFAU, K.O., LEPARD, O.L. & BARTLETT, J.W. Comparison of the incidence of udder infection and mastitis in two cow families. Cornell Vet., 34, 185-192, 1944.

80. NEWMAN, R.W. A one-solution technique for the direct microscopic method of counting bacteria in milk. Monthly Bul. of Calif. Dept. of Agric., 16, No. 1, 1-7, 1927.

81. PETERSON, E.H. & HASTINGS, E.G. A study of the possible relationship between nonspecific mastitis and streptococcic infection of the bovine udder. Cornell Vet., 29, 11-24, 1939.

82. PETERSON, E.H., HASTINGS, E.G. & HADLEY, F.B. The pathology of nonspecific mastitis and consideration of possible etiological agents. Cornell Vet., 28, 307-324, 1938.

83. PLASTRIDGE, W.N., ANDERSON, E.O. & WEIRETHER, F.J. Infectious bovine mastitis. 8. The control of Streptococcus agalactiae mastitis by a segregation program based on periodic laboratory tests. Storrs Agric. Exp. Sta. Bul. 240, 54 pp., 1942.

84. PLASTRIDGE, W.N., ANDERSON, E.O., WILLIAMS, L.F. & WEIRETHER, F.J. Infectious bovine mastitis. 7. Characteristics of udder staphylococci. Storrs Agric. Exp. Sta. Bul. 231, 60 pp., 1939.

85. POUNDEN, W.D. Deferred milking - a method of handling acute streptococcic mastitis cases. Cornell Vet., 31, 339-344, 1941.

86. POUNDEN, W.D. & JOHNSON, M.M. The influence of an udder coccus upon the activity of mastitis streptococci in milk. Am. J. Vet. Res., 2, 317-318, 1941.

87. ROSELL, J.M. Laboratory and field methods for the detection of mastitis. Canad. Pub. Hlth. J., 25, 124-130, 1934.

88. ROSELL, J.M. The most practical field and laboratory tests for detection of mastitis. Sci. Agric., 15, 169-175, 1934.

89. SANDERS, D.A. Musca domestica a vector of bovine mastitis (Preliminary Report). J. Am. Vet. Med. Ass'n, 97, 120-123, 1940.

90. SANDERS, D.A. Hippelates flies as vectors of bovine mastitis (Preliminary Report). J. Am. Vet. Med. Ass'n, 97, 306-308, 1940.

91. SCHALM, O.W. Recommendations for the use of penicillin in the treatment of bovine mastitis. Bul. Cutter Laboratories, Berkeley, Calif.

92. SCHALM, O.W. The treatment of streptococcic mastitis by infusion of the udder with entozon. J. Am. Vet. Med. Ass'n, 47, 20-27, 1940.

93. SCHALM, O.W. The use of trypaflavin in the infusion therapy of streptococcic mastitis. Am. J. Vet. Res., 2, 117-126, 1941.

94. SCHALM, O.W. The treatment of chronic bovine mastitis. J. Am. Vet. Med. Ass'n, 50, 323-334, 1942.

95. SCHALM, O.W. Treatment of chronic mastitis during the dry period. J. Am. Vet. Med. Ass'n, 54, 78-83, 1944.

96. SCHALM, O.W. Gangrenous mastitis in dairy cows. N. Am. Vet., 39, 279-284, 1944.

97. SCHALM, O.W. & MEAD, S.W. The effect of incomplete milking on chronic mastitis caused by Streptococcus agalactiae. J. Dairy Sci., 26, 823-832, 1943.

98. SCHÖNBERG, F. Zum Einfluss des Gelben Galts auf die Gelbfluoreszenz der Kuhmilch. Z. Fleisch - u. Milchhyg., 53 (22), 215-216, 1943. Via Dairy Sci. Abstr., 7, 1945, p. 57.

99. SEELEY, H.W., ANDERSON, E.O. & PLASTRIDGE, W.N. Action of penicillin against mastitis organisms in milk. J. Dairy Sci., 28, 887-891, 1945.

100. SHARP, P.F. & STRUBLE, E.B. Period of lactation and the direct titratable chloride value of milk. J. Dairy Sci., 18, 527-538, 1935.

101. SHAW, A.O. & BEAM, A.L. The effect of mastitis upon milk production. J. Dairy Sci., 18, 353-357, 1935.

102. SHERMAN, J.M. The streptococci. Bact. Rev., 1, 1-97, 1937.

103. SOMMER, H.H. & MATSEN, H. The relation of mastitis to rennet coagulability and curd strength of milk. J. Dairy Sci., 18, 741-749, 1935.

104. STEBBINS, E.L., INGRAHAM, H.S. & REED, E.A. Milk-borne streptococcic infections. Am. J. Pub. Hlth., N.S. 27, 1259-66, 1937.

105. SWETT, W.W., GRAVES, R.R., MATTHEWS, C.A., CONE, J.F., & UNDERWOOD, P.C. A study of the effectiveness of sulfonamide preparations in the elimination of bovine mastitis. U.S.D.A. Tech. Bul. No. 884, 20 pp., 1944.

106. UDALL, D.H. & JOHNSON, S.D. The diagnosis and control of bovine mastitis. Cornell Agric. Exp. Sta. Bul. 579, 15 pp., 1933.

107. WASHBURN, L.E. Fasting of dairy cattle shows promise as a management method for control of mastitis. Col. Agric. Exp. Sta., Farm Bul., 5, No. 3, 3 pp., 1943.

108. WATTS, P.S. Field observations on the control of mastitis. Proc. Soc. Agric. Bact. (Gt. Br.), 1942, p. 35. Abstr. J. Dairy Sci., 26, p. A128, 1943.

109. WELCH, R.C. & DOAN, F.J. The heat stability of evaporated milk made from hard-curd milk, soft-curd milk and milk from mastitis infected udders. J. Dairy Sci., 18, 287-294, 1935.

110. WESCHE, G. Tierarztl. Rdsch., 39, 478- , 1933. Via biennial reviews of the progress of dairy science, Section E. The diseases of dairy cattle. I. Mastitis. J. Dairy Res., 7, 291-299, 1936.

111. WHITE, G.C., COUTURE, G.W., ANDERSON, E.O., JOHNSON, R.E., PLASTRIDGE, W.N. & WEIRETHER, F.J. Chronic bovine mastitis and milk yield. J. Dairy Sci., 20, 171-180, 1937.

112. WIEDMANN, F. Z. Untersuch. Lebensmitt., 63, 113- , 1932. Via biennial reviews of the progress of dairy science, Section E. The diseases of dairy cattle. I. Mastitis. J. Dairy Res., 7, 291-299, 1936.

113. YALE, M.W. & MARQUARDT, J.C. Factors affecting the survival of Streptococcus pyogenes in cheese. J. Milk Tech., 3, 326-333, 1940.

ACKNOWLEDGMENT.

The author is deeply indebted to the British Columbia Industrial and Scientific Research Council for permission to use the material contained herein as a thesis for the Degree of Master of Science in Agriculture at the University of British Columbia. The work was undertaken as a Research Project of the Council under its Sub-Committee on mastitis with Dr. J. G. Jervis as chairman.

The author also wishes to express his sincere appreciation to Dr. B. A. Eagles, Head of the Department of Dairying at the University of British Columbia, for his continued guidance and encouragement during the course of this study, and for his assistance in the preparation of this paper.

The assistance of the following persons in the laboratory and statistical work pertaining to the studies reported herein is gratefully acknowledged: Dr. S. N. Wood, Mr. Vernon H. Grigg, Miss Sylvia R. Hall, Miss Lene Lourie, Mrs. M. Kathryn Hall, Mr. Ian J. McDonald and Mr. Frederick D. Smith.
