A Study of Somatic Cell Concentrations in Milk of Laboratory Mice

by

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(ABSTRACT)

A bidirectional selection experiment for increased and decreased somatic cell counts (SCC) in milk was conducted with two selected lines (high line or HSCC, low line or LSCC) and one control line (CSCC) of mice.

Distribution of milk SCC in mice was 4 to 5 times the distribution in Holsteins. The shape of the lactation curve of mice was similar to that of Holsteins with maximum yield at day 7 (2.06 gms). The phenotypic regression of lactation milk yield on lactation milk SCC (-0.276) was significant (P < .05) and indicated that dams with higher SCC produce less milk. Small phenotypic correlations of milk SCC with blood SCC (-0.09) and percentage phagocytic cells (-0.06) were found, indicating that these traits are unrelated. A small but significant (P < .05) negative correlation (-0.14), was found between response to endotoxin challenge and milk SCC indicating that dams with inherently higher milk SCC responded less to endotoxin challenge than dams with lower milk SCC. No major pathogens which cause mastitis in cattle were detected in milk of mice. In addition, the bacteria identified (Bacillus sp., Corynebacterium sp. etc) did not cause any serious infections and/or increase in milk SCC in mice.

Selection for high and low milk SCC produced a symmetrical response in the two selected lines (HSCC and LSCC), such that after 7 generations of selection, the two lines differed by more than 500,000 cells/ml of milk. A small negative genetic regression (-0.162) of milk yield on milk SCC suggested a small correlated response in yield opposite in direction from the direct response for milk.
Small negative genetic regressions of blood SCC and percentage phagocytic cells on milk SCC (-0.087 and -3.492) suggested that these three traits are genetically independent. Selection on milk SCC did not result in change either in total leukocytic cells per ml of blood or in percentage phagocytic leukocytes in blood. A negative genetic regression of response to challenge on milk SCC (-3.201) was found suggesting that selection for low milk SCC results in an increase of the ability of the individual mouse to elevate milk SCC after an injection with E. coli endotoxin. However, more data are needed to confirm this conclusion. Phenotypic correlations between milk SCC and several measures of fitness and genetic regressions of these measures on milk SCC were negligible.
Dedicated to my lovely daughter Sonia
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Introduction

Mastitis is an inflammation of the mammary gland which is characterized by physical and chemical changes of the milk as expressed by the increased occurrence of somatic cells, especially leukocytes (170). Other signs of the disease are pathological changes of the mammary gland itself. The disease may develop clinically in an acute, subacute or chronic form. Common to all these forms is the main symptom of an increased cell count which is a response to the cause of irritation, mostly pathogenic microorganisms.

Blosser (16) estimated that mastitis costs $117.35 annually for each cow in the United States. Yearly costs per cow were 386 kg of discarded milk, veterinary costs $1.97, drugs $3.86, labor $2.28, and decreased sale value per cow $5.72. Overall annual losses in the U.S. were estimated by the same author at $1.3 billion. In addition, it has been projected (96) that approximately one-half the quarters of one-half the cows are infected at any given time, with about 20% of these exhibiting clinical mastitis, characterized by abnormal milk and obvious udder inflammation. However, sub-clinical cases appear to be responsible for more than half the economic losses (39) and over 90% of the cases (58).

Efforts to minimize losses from mastitis currently involve on farm hygiene, proper installation and maintenance of milking equipment, disinfection of teats following milking, antibiotic treatment of
udders and culling of chronically infected cows (111). Although research has demonstrated the efficacy of these procedures, no long-term reduction in mastitis incidence through their use has been documented (180). Additionally, the current liberal use of antibiotics by U.S. dairymen, even if not restricted in the near future, can be expected to result in reduced cure rates (144).

Mastitis long has been considered entirely a management problem. Hence, there has been no documented effort to select for genetic resistance to mastitis in dairy cattle (151). Genetic correlations measure the association between breeding values of two traits and indicate the extent to which selection for one trait will produce a genetic change in the other trait (156). Recent reports suggest a positive genetic correlation between milk yield and susceptibility to mastitis (59, 80, 117, 178), indicating control of mastitis may become more difficult.

These factors argue strongly for a program of selective breeding which will increase resistance of dairy cattle to mastitis infections. Although the desirability of breeding dairy cattle more resistant to mastitis has been recognized for many years, the ability to do so has eluded both researchers and breeders alike. Due to constraints imposed by the nature of the disease and the structure of breeding populations in dairy cattle, attaining genetic resistance to mastitis requires accurate genetic evaluations for resistance of the sires available to dairymen through artificial insemination. Since resistance is sex-limited (measured only in females), genetic evaluation of sires requires an assessment of mastitis incidence for their daughters. Two direct measures of mastitis have been considered: 1) incidence of clinical cases of mastitis, 2) bacteriological test results. Neither of these measures are feasible traits for selection for decreased incidence of mastitis (101). Direct measurement of clinical cases of mastitis is cost efficient but has a serious disadvantage of not detecting subclinical cases which are reported to be responsible for over half the economic losses and more than 90% of the cases. Furthermore, the cost of obtaining periodic bacteriological cultures on large numbers of cows would be prohibitive.

Therefore, the major impediment to genetic evaluation and selection for mastitis resistance has been the lack of a cost-effective measure of clinical and subclinical mastitis for the large number of
A measure which potentially can satisfy this requirement is milk somatic cell count (SCC) (101). This measure is cost effective since it can be made by automated electronic cell-counting devices on milk samples which currently are collected (through Dairy Herd Improvement associations) for determining fat and protein content of milk. Further, SCC is an indirect measure of mastitis incidence in that an elevation of SCC accompanies both clinical and subclinical bacterial infections of the udder. Thus, SCC can provide an indirect indicator of mastitis infection on large numbers of cows at relatively little additional cost to dairymen.

But even in terms of expected response to selection, SCC appear to be a better parameter than clinical measures. Heritability is the proportion of the phenotypic variance which is due to additive genetic variance. It measures the importance of genetics as a cause of differences among individual phenotypes. Also, heritability is the portion of the difference between phenotypes of two individuals which is, on average, due to the difference in their breeding values (156). In a recent review (156) it was concluded that heritability is higher for sec (about .17) than for clinical measures of mastitis (about .12). Of course improving mastitis resistance through selection for SCC depends mainly on the genetic relationship between SCC and clinical mastitis (172).

The major unanswered question with regard to SCC involves the proper interpretation and use of measured differences in SCC among cows, and among daughter averages for sires. The neutrophils (PMN) of SCC are phagocytic cells which represent a principal defence mechanism of the cow against invading bacterial organisms (4). These cells increase dramatically following infection and primarily are responsible for elevated SCC in mastitis. The question of critical importance is whether differences in SCC reflect differences among cows in their abilities to respond to bacterial organisms in the udder, or differences in their abilities to preclude organisms from the udder. Given the former interpretation, we desire and should select for increased SCC in our dairy populations. However, if the latter interpretation is correct, selection should be for decreased SCC. No consensus exists on this question. It has, in fact, been suggested that selection for decreased SCC might yield a reduction in mastitis incidence in early generations of selection, but later produce an increase
in mastitis problems by reducing the abilities of cows to respond to bacterial infection (22). It would seem, therefore, that the correct biological interpretation of differences among animals in SCC can be determined only by observing the results of divergent selection for increased and decreased SCC. Since such a project would have been prohibitively expensive and time-consuming with cattle, it was proposed to use the laboratory mouse as a pilot organism.

The objectives of this experiment were:

1. To develop, through single trait selection, lines of laboratory mice which are genetically divergent for somatic cell concentration in milk.

2. To estimate correlated responses to selection, and characterize lines genetically diverse for SCC in milk, with respect to:

   a. milk yield,

   b. circulating (blood) leukocyte pools, and composition of leukocyte pools (neutrophils, monocytes, and others),

   c. ability to mobilize somatic cells in response to chemotactic challenge of the mammary gland,

   d. general fitness and reproductive performance, and

   e. natural infection rates of the mammary gland.
Review of Literature

Economic Losses due to Mastitis

Investigators (40, 73) have classified economic losses from mastitis into the following categories: a) realized (clinical) and unrealized (subclinical) reduced milk production; b) milk discarded because it contained antibiotics or was of abnormal composition; c) cost of veterinary services to treat acute and chronic mastitis; d) cost of drugs purchased by dairymen for intramammary infusion; e) cost of increased labor to care for mastitic cows; f) decreased sale value of cows sold for dairy purposes; g) increased herd replacement costs when cows were culled because of mastitis. The largest of these losses was that caused by reduced milk production.

Researchers recently have made attempts to express losses in milk production due to mastitis in conjunction with measuring and reporting the SCC of milk. In a 3-yr study of 108 lactations of Holstein cows from the N. Mexico State University herd Miller (94) reported that clinical mastitis reduced milk production more than 20% for the total lactation. Cows producing milk with over 500,000 SCC/ml were considered to have clinical mastitis. Janzen (73) cited losses of milk per quarter per day in mastitic cows of .34 to 2.66 kg. Ward and Schultz (175) examined milk from 874 quarters with the filter-DNA method. They reported losses of 15% from quarters with SCC
of 2 million/ml and more than 25% from quarters with SCC of 4 million/ml. In another study
Schultz (142) compared milk production for full lactations of cows whose milk gave varying re-
actions to monthly test by the filter- DNA method. Cows whose milk averaged less than 500,000
somatic cells/ ml produced 572 kg more milk and 25 kg more fat per lactation than cows whose
average lactation SCC was more than 1 million/ml. King (81) cited a Milk Marketing Board study
in Great Britain in 1971 which attributed losses of 164, 289, 661, and 770 kg of milk/cow per yr to
SCC ranges of 250,000 to 499,000; 500,000 to 749,000; 750,000 to 999,000; and 1,000,000 and
over/ml.

In addition to losses due to reduced milk production there is also the loss from discarded milk due
to treatment with antibiotics. Natzke (108) estimated discarded milk losses as 27 kg/day for 5 days
for each clinical case and 1 to 1.5 clinical cases/cow per yr in herds without teat dip and dry cow
therapy programs. Also Dobbins (40) estimated the losses from discarded milk as 34 kg or
$7.72/cow per yr in 31 random herds involved in the Georgia Quality Milk program. The cost of
veterinary services due to mastitis has been estimated by the same author to be $7.20 /cow per yr.
He also estimated the cost of drugs to be $2.16/cow per yr. because of mastitis additional labor. Finally,
Dobbins reported an increase of $1.50/cow per yr. because of mastitis additional labor. Finally,
Dobbins estimated a replacement cost due to mastitis of $39.00/cow per yr. in these 31 Georgia
herds.

Obviously the total losses of mastitis are enormous. Nielsen (116) calculated the total annual loss
in the United States in 1976 as $600 to $800 million. Blosser (16) in a 1976 survey from 33 States
representing 9.5 million cows or 86% of the dairy cow population found that total losses caused
by mastitis were $117.35/cow per yr. or about $1.3 billion for all cows included in the survey. He
also reported that if economic losses from mastitis were reduced 2% per year for 10 yr. by research
and expenditures for mastitis research remained the same, the benefit to cost ratio from mastitis
research would be about 9.6 to 1. Finally in a more recent study Miller (95) estimated the total
economic losses due to mastitis at $2 billion annually.
Etiology of Mastitis-Conventional Control Methods

In contrast to the classical diseases of cattle most of which are produced by a specific agent, numerous microorganisms- bacteria, molds, yeasts, and possibly other agents- may be involved in the development of mastitis. Streptococci and coagulase-positive Staphylococci, Escherichia coli and Corynebacterium pyogenes are most frequently detected (170). Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis are the most common strains of Streptococci isolated from mastitic milk. In addition, Staphylococcus aureus and Staphylococcus epidermidis are the most common mastitis Staphylococci (170). Finally, another coliform agent found in abnormal milk (in addition to E. coli) is Klebsiella pneumoniae.

According to Tolle (170) the microorganisms may reach the tissue of the mammary gland through a variety of pathways- the skin, the blood, the lymph. Normally, however, the infection is exogenous and penetrates the udder through the streak canal. In addition, the mastitis infection process is influenced by the presence, numbers, pathogenicity, virulence and antigenicity of the pathogens in the environment of the cow.

Until now, Streptococcus agalactiae has remained one of the most frequently detected agents of bovine mastitis (170). According to international literature the proportion of isolations, related to the total number of samples studied, ranges between 0.4 and 6.9%; the proportion related to the total number of mastitis streptococci isolated from the udder varies between 23 and 85% (65). This bacterial species is, at the same time, increasingly isolated from cases of severe human disease during recent years.

Tolle (170) reported that more than 90% of mastitis infections are produced by Staph. aureus, Str. agalactiae, Str. dysgalactiae and Str. uberis. He also indicated that it is possible to eradicate Str. agalactiae completely since this organism may survive in the environment only to a limited extent and it is sensitive to penicillin. Eradication of Staph. aureus may be possible in principle, but ap-
pears to be much more difficult as the agent is able to penetrate the tissue and to surround itself with reaction products. Str. uberis is widespread in dairy herds and outside the udder (37). Even Str. dysgalactiae is widespread in nature, occuring in many animals, and is regularly found in the tonsils and vagina of the cow. E. coli and other Enterobacteriaceae, C. pyogenes and Ps. aeruginosa contribute to the remaining 10% of infections (170). As a rule, C. pyogenes is dependent on the presence of concurrent infections to produce clinical mastitis. The others are widespread in water and the environment of the cows (dung, utensils) and there is no chance of eliminating these sources of infection. The same author (170) concludes that mastitis cannot be eradicated completely; it may only be reduced by systematic monitoring and sanitation programmes.

A considerable amount of research has been undertaken to control mastitis in dairy populations through prophylactic and therapeutic procedures. Prior to 1960 many trials tested the value of individual hygienic practices in reducing bacterial populations (43, 82). These practices included the use of sanitizers in wash water, individual towels, flushing of liners, and germicides on teats. Each of these procedures reduced numbers of bacteria, but their effectiveness in reducing new mastitis infections remained unknown; therefore, their value in mastitis control only could be speculated.

The major breakthrough in mastitis control occurred when Neave, Dodd, and coworkers from the National Institute for Research in Dairying combined and tested the value of these hygiene practices in a series of well-controlled field studies (42). Two hygiene levels were compared: 1) a complete hygiene system that consisted of wearing rubber gloves dipped in sanitizing solution before touching the next cow, pasteurizing teat cup clusters between cows, washing udders with single service towels dipped in sanitizer solution, and dipping teats in a germicidal solution after milking, and 2) a system of no hygiene. They showed that in the absence of hygiene an average of two new infections occurred per cow per year and that with complete hygiene during lactation this could be reduced to one new infection per cow per year. However, even with the 50% reduction in the new infection rate, little progress was made in reducing the percentage of quarters infected. They concluded that for maximum gain in mastitis control one must not only reduce the rate of new infection but also the duration of infection.
The importance of the dry period with respect to both initiation and duration of new infection was demonstrated in an early study (111). Cloxacillin carried by two different vehicles was infused at the time of drying off. A comparison at the next calving revealed that quarters treated with the antibiotic in a long-acting base had fewer persisting infections and developed fewer new infection than quarters treated with the antibiotic in a quick-release base.

In another study (177) the effective hygiene practices of (42) were combined with routine dry cow treatment in an attempt to couple decreased infection rate with reduced duration of infection. The 3-yr study was initiated on 32 commercial herds in England and 27 in New York (Cornell University) (109). Infection was decreased an average of 75% in the herds in both countries. Comparable field studies in Australia and Israel have similar results (104, 184). These studies have formed the basis for the presently recommended mastitis control procedures of regular use of a teat dip combined with dry cow therapy.

Dry cow therapy is presently the most common means for eliminating infections in a mastitis control program. Another value of dry cow therapy is derived from prevention of new infection (26). However, the extensive use of antibiotics in disease prevention is of concern (111). According to Levy (86) there is a general agreement that antibiotic use is responsible for the rise in resistant bacteria, but controversy continues over whether antibiotic use in people or animals has contributed most to the environmental pool. This issue is complicated by the facts that both groups receive the same kinds of antibiotics and each receives about half of the more than 35 million pounds of antibiotics produced in the U.S per year. However, the environments of animals and human beings are not separate. Exchange of bacteria occurs between both groups. Bacteria originating in the animal gut can find their way into the human intestinal tract. When pathogenic, they can cause human disease.

Lactation therapy often is looked upon unfavorably, not only because its effectiveness is inferior as compared to therapy in the dry period but also because of the economic losses associated with discarded milk (111).
Another procedure, routine culling not only removes cows with a high percentage of days infected from the herd, but also brings into the herd replacement animals in first lactation that have a low percentage of infected quarters. While culling aids in reducing herd infection, increasing the culling rate as a means of reducing mastitis is seldom warranted economically (112).

There are also several management and environmental factors related to resistance to infection but usually not considered as control procedures. These include the prevention of teat injury, proper sanitary care of teat cannulae and treatment materials, and reduction of stress (111).

The final route to follow if one wishes to provide a complete or near complete control program is to decrease our animals' susceptibility to mastitis. The goal of most disease control programs is to increase the number of resistant individuals in the population. This might be accomplished by inducing resistance or by selection of those naturally resistant (111).

In conclusion then, mastitis is caused by several microorganisms. Some of these are transmitted from cow to cow (Staph. aureus, Strep. agalactiae). Others are transmitted to the cow from the environment (Streptococci other than agalactiae, Coliforms, Corynebacteria etc.). The contagious bacteria are easier to detect and eliminate mainly through antibiotics. However, the excessive use of these antibiotics is looked upon unfavorably by many scientists. Most of the environmental bacteria are difficult to detect and eliminate with present control methods. Therefore, the need for looking at alternatives of meeting the mastitis problem has recently become more obvious. One of these alternatives would be to select for individuals with increased genetic resistance to mastitis.

Defense Mechanisms

If we were to select our dairy populations for increased resistance to mastitis, we should direct our efforts towards increasing the effectiveness of one or more of the cow's natural defence mechanisms.
Reiter and Bramley (130) have distinguished among the following defense mechanisms of the udder: the teat duct, phagocytosis, lactoferrin, and the lactoperoxidase/thiocyanate/peroxide systems. In addition, Miller (96) reviewed the role of the teat opening as a defense mechanism. He stated that the times and degrees to which the teat end is open depend upon the relaxation and contraction of the sphincter muscle around it. The sphincter also influences milk removal and, therefore, there may be a conflict between mastitis resistance and rapid milk removal.

Two papers (2, 107) provide the key to an understanding of the defensive role of the duct against infection; they give a histological description of the duct and demonstrate the temporary breakdown of the resistance of the duct following partial removal of the keratin. More recently it has been shown that removal of keratin not only increases penetration but also facilitates the multiplication of Strep. dysgalactiae and Klebsiella pneumoniae in the duct. (20). Additional research (1) has demonstrated that lipids and a basic protein extracted from keratin are bacteriostatic or bactericidal. While there is some evidence that the lipid content varies between susceptible and resistant ducts (171), a similar association has not yet been shown for basic proteins. Recently, Miller (96) reported that researchers have postulated that the sebaceous cells in the teat canal secrete a sebum-like product which has antibacterial properties. Finally, Stavikova and Lojda (165) reported a fairly high heritability of lipid composition of keratin, which suggests that the keratin properties may represent a heritable defense mechanism.

One of the most important aspects of defense mechanisms is phagocytosis. Paape et al. (124) refer to phagocytosis as the process of recognition, ingestion, and digestion of foreign particles (bacteria, necrotic tissue, etc.) by leukocytes. The majority of leukocytes found in milk are polymorphonuclear leukocytes or neutrophils (PMN) that enter the mammary gland from blood via diapedesis, and function by phagocytosing bacteria and killing them intracellularly (115). The PMN leukocytes make up most of the leukocytes in circulating blood of many animal species. However, in the bovine, PMN make up only 25% of the total leukocyte count (138). Nevertheless, when the size (avg 682 kg) and blood volume (8% body weight) of mature lactating Holstein cows are considered, a potential pool of over 100 billion circulating PMN are present to fight infection.
The protective role of the PMN in the lactating udder was shown by Jain et al. (72), who produced neutropenia in cows by injecting them with equine anti-bovine leukocyte serum. When udders were infected experimentally with Aerobacter aerogenes, the neutropenia led to unrestricted multiplication of the coliform within the infected quarters. In a similar study by Schalm et al. (139) udders of cows chronically infected with Staphylococcus aureus became gangrenous when neutropenia was produced with equine anti-bovine leukocyte serum.

Phagocytic competence on a systemic level is composed of two elements: ability of an individual cell to phagocytose and total number of cells available. Associated with cell number is the rapidity of mobilization of PMN into the gland after entry of organisms (101). Paape et al. (124) discussed factors affecting phagocytic ability of the individual cell in the mammary gland and the fact that individual cell competence is lowered after it leaves the circulation. Factors influencing competence were glycogen depletion, lack of opsonins, ingestion of milk fat and casein particles and pH level of phagocytic vacuoles. In addition, he reported that cows differed in ability of their milk PMN to phagocytose, but quarters of the same cow did not differ. Earlier studies on such cow variation were by Newbould (114) and Wisniowski et al. (179). While several factors could cause these cow differences, genetic variation is the most probable (101). Furthermore, there are results from experiments with laboratory animals (which will be discussed in a latter section) further suggesting possible genetic control over phagocytic functions.

The PMN are certainly the most important phagocytic cells. However, they are not the only ones. The total cell population of mammary secretion includes macrophages and lymphocytes in addition to PMN, with epithelial cells representing less than 2% of the total SCC (70). All of these leukocyte types probably play a role in udder defense. PMN were found to decrease from late lactation to involution, while macrophages and lymphocytes increased (115).

The final defense mechanism against intramammary infections that the cow possesses is its immune response system. Miller and Schultze (101) have subdivided this system into Humoral and Cell Mediated Immunity. Humoral immunity refers to the role of immunoglobulins (Ig) secreted by
certain lymphocytes. Actions of three Igs (IgA, IgM, and IgG) include opsonization, complement fixation and neutralization of toxins. Results of these actions include the prevention of the adherence of bacteria to host tissue and agglutination of pathogens. Opsonization is the process by which IgG (G1 and G2) enhances ingestion of pathogens by the phagocytes, so immune response is also related to the phagocytic defense mechanism (101). In addition, Cell Mediated Immunity is a specific immunity which is accomplished by small lymphocytes. It is responsible for reactions such as allograft rejection, delayed hypersensitivity and tuberculin test reactions. In this response, the antigen combines with a subpopulation of the lymphocytes. As a result lymphokines are produced which lead to local inflammatory reactions (leukocytosis) and promote the removal of foreign substances (101). Recent results (115) of phagocytosis assays indicated that IgM was the most important isotype for promoting phagocytosis followed by IgG2 and IgA. Similarly Hill (69) found that opsonic activity of normal milk is associated with the IgM fraction; however, this activity was associated with IgM and IgG2 in hyperimmune cows.

Finally, the role of some non-antibody factors called Lactenins has been discussed by some researchers. These factors include the Lactoferrin and the lactoperoxidase/thiocyanate/hydrogen peroxide systems (131). Lactoferrin (Lf) is a minor whey protein of milk that functions by binding iron, making it unavailable to bacteria that require it for growth. Coliform growth, for instance, was found to decrease 10-to 100,000-fold as involution progressed with concomitant increases in Lf (161). However, Lactoferrin and antibody had little effect on streptococci. Finally, the Lactoperoxidase system was found to be capable of killing bacteria such as E. coli, Pseudomonas aeruginosa, Salmonella typhimurium and Staph. aureus (130).

Virtually nothing is known of the genetic control of these defenses in the bovine (101). However, there is a great deal of information in other laboratory species. It has been found, for instance, that at least three sets of genes are involved in control of humoral immunity in mice. First, a set of structural genes controls the coding for amino acid sequence of the Ig molecules on B-cells. Secondly, a set of independent autosomal genes controls the quantitative function of the antibody-secreting plasma cells (101). Also, French workers selected mice for high and low response to sheep
red blood cells (15). They found that a set of 10 independent loci were involved in quantitative response to the antigen. Another set of genes regulates aspects of antibody function such as specificity and affinity. Katz and Steward (79) selected mice for high and low affinity of antibodies to a specific antigen. These genes were independent of those controlling antibody quantity. In addition, at least two sets of genes control cell-mediated immunity. There is a set of structural genes which code for the composition of T-cell receptors (the main function of T-cell receptors is the antigen recognition). There is also a set of immune response regulatory genes which governs the antigen recognition function of T-cells. These genes have been identified in many species and have been shown to be linked with histocompatibility genes (101). Finally, there are reports on genetic control of the function of Macrophages which in addition to their phagocytic properties they also have immune properties (101).

In summary, various defense mechanisms operate in the bovine mammary gland to prevent penetration of bacteria, suppress growth of microorganisms, and eliminate bacteria from the udder. The teat canal provides the first barrier to mastitis pathogens. This structure is a fairly good defense mechanism; however, some bacteria are able to gain access to the interior spaces of the udder where milk leukocytes serve as the second line of defense. PMN cells are probably the most important phagocytic cells. These leukocytes are primarily the ones which increase largely in number after an infection has been established and these cells are also the ones which are found in large numbers in SCC determinations. In addition to PMN the macrophages are also known to possess both phagocytic properties as well as immune properties. The final defense mechanism is the immune response. Aspects of immune response include: immunoglobulins, cell mediated immunity, and lactoferrin. IgM appears to be the most important isotype promoting phagocytosis followed by IgG2. Lymphokines which are produced in the cell mediated immunity are also an important aspect of defense. Finally, lactoferrin, the most important of the Lactenins, is highly effective in inhibiting coliform growth during mammary involution, but it has little effect on Streptococci. Genetic factors are responsible at least to some extent for the variation among individuals with regard to several types of defense mechanisms. Such variation includes differences among individuals.

Review of Literature
in the size of the teat duct, lipid composition of keratin, numbers and phagocytic ability of leukocytes, and aspects of immune response.

SCC as a Measure of Genetic Resistance to Mastitis

During the past 15 years, genetic improvement for the yields of milk and milk components has progressed at an unprecedented rate. Currently, genetic gain for milk yield is estimated at 100 to 130 pounds per year and accounts for 50 to 60% of the improvement in annual yield per cow (156). A question that needs to be answered is: What is the effect of intensive selection for yield on genetic resistance to mastitis? Genetic correlations between milk yield and mastitis have been estimated as .30, .33, and .44 by Wilton, et al. (178), Miller, et al. (98) and O’Blleness, et al. (117). Simulation studies by Philipsson, et al. (125) and Strandberg and Shook (166), both assuming a genetic correlation of .30 found that selection for yield is accompanied by an increase, respectively, of .4% in mastitis incidence and .02 mastitis cases per year. The evidence is quite clear that genetic improvement for yield is causing a slow increase in mastitis incidence. It is imperative that the animal breeding industry include mastitis in breeding decisions (156).

Selection of a Genetic Measure of Resistance: A definition of genetic resistance to mastitis is given by Miller and Schultze (101). They state that "we mean some innate physiological defense mechanism of the animal, which enables it to prevent pathogens from establishing an infection. The mechanism which confers a degree of resistance is present in the cow even before she is exposed, but can only be expressed in response to bacterial exposure". If a genetic basis exists for resistance or susceptibility to mastitis, the majority of the researchers agree that not a single locus but many loci are probably responsible for such a complicated trait (101). Then after we find an accurate estimate of resistance we should turn to a statistical appraisal of a group of animals to predict which animals possess the most favorable genes.
Miller (96) has reviewed several measures of resistance to mastitis as well as the possibility of using one or more of these parameters in selective breeding programs. Repetitive bacteriological testing of all quarters perhaps can be considered the most accurate measure because it indicates both clinical and subclinical aspects of the disease. Test results also provide species information essential for mastitis control. However, effective bacteriological testing requires periodic quarter milk samples from all cows in the herd. Frequency of sample collection can be debated, but monthly is probably a minimum for management information. Because normal Dairy Herd Improvement (DHI) milk samples are composite samples (from all quarters) and thus not suitable for standard bacteriological testing, routine field testing is not at all likely under U.S. conditions (156). In addition, the high cost of bacterial culturing eliminates it from consideration in breeding programs (96). Heritabilities for bacteriological tests have been reviewed by Miller (96). The estimates range from about zero to .20 with a mean heritability of .11.

Another direct criterion of mastitis is the recording of each clinical case, i.e., antibiotic treatment. Perhaps the greatest advantage of clinical mastitis is the low cost of recording as no milk sample or laboratory assay procedures are required (156). However, there is a large drawback of this method and this is that we fail to detect the subclinical infections which according to Giesecke (58) represent 93-97% of all mastitis cases. Also, few producers are willing to routinely record the events which constitute clinical mastitis (101). It also would be difficult to achieve a standard definition for clinical mastitis throughout the industry and impossible to apply that definition consistently to every cow in every herd. Finally, clinical cases do not represent mastitis on a continuum but rather as a condition which is present or absent. Surely, a continuous or quantitative representation of mastitis is proper because it exists in subclinical or clinical form, with duration from a few days to several months, and mild to extreme severity (156). Heritabilities of clinical cases have also been reviewed by Miller (96). Estimates range from -.03 to .50 with a mean value of .12. Young et al. (183) have reported on the genetic correlation between bacteriology and clinical cases. This correlation was found to be .29.
If we decide that bacteriological tests are too expensive to apply and clinical cases are impractical to use in selective breeding programs, the next category of measures to examine involves screening tests based on various milk properties. Although these tests are aimed at detecting the disease state rather than resistance, some of them could also be used for genetic evaluations if their $h^2$ was found to be reasonably high and if their relationship to mastitis incidence or resistance was high. The most important such test is milk SCC (96). The determination of cell content is being done either through microscopic counting (DMSCC) or through electronic particle counting. The method of microscopic counting of cells on a dry stained smear has been available since the beginning of the century. Even today, the method of Prescott and Breed (127) is considered the reference method of other tests. In recent years, automation of cell counting has been developed. Instruments such as Coulter counter, Fossomatic and others are being used widely in milk laboratories for SCC determination. Mastitis control systems in many developed countries are heavily based on SCC reports issued by such laboratories (41). In general, correlations measure the degree of association between two variables. Correlations between direct microscopic and electronic counting run higher than .90 (88). On the other hand as many as 1500 samples per day per instrument is possible today by using instruments such as the Fossomatic counter for instance (88). In addition to the direct methods of SCC determination there are a number of biochemical tests which are indirect measures of SCC. These tests include California Mastitis Test (CMT), Wisconsin Mastitis Test (WMT), Whiteside Test, etc. Their correlation to direct methods of SCC is also high (> .90) (101).

Finally, there are some other screening tests in addition to SCC. Some of these tests that have been examined by researchers are: lactose content (132), mineral content (141), lactoferrin (56), lysozyme content (149), and lactoperoxidase content (83).

It seems that the most suitable parameter, from an economic point of view, to use in sire summaries at the present time is milk SCC. Somatic cell testing was introduced in DHI programs in the mid 1970s. Currently more than 2.4 million cows, representing 50% of all DHI cows, are on somatic cell test. This extensive data base in DHI makes possible, at low marginal cost, the genetic evaluation of bulls and cows on a national basis (156). However, before any use of SCC in genetic...
programs is made, a definite relationship between mastitis resistance and cell count should be established (96).

The bearing of SCC to mastitis is that, accompanying both clinical and subclinical infections, within 24 h an elevation of SCC of milk occurs, although this elevation is less if there is no obvious inflammation. At the same time, a change occurs in the relative proportions of the several types of cells in milk (96) with PMN cells increasing in number more than other leukocytes. Table 1 gives the proportions of different cells in milk from uninfected udders. It was mentioned before that PMN and macrophages are the most important phagocytic cells. However, lymphocytes are also involved in the animal’s immune response by influencing the function of the PMN (101).

In general, the role of cells in milk as defense against mastitis causing organisms presents some problems in the interpretation and use of SCC in mastitis control. Eberhart et al. (47) found that while mean cell counts of infected and uninfected cows are quite different, the distribution of counts overlap substantially and therefore an absolute cell count value accurately discriminating between infected and not infected cannot be specified. The difficulty in determining the precision with which cell counts predict infection status is due to the ‘normal’ inherent animal to animal variation in cell count and the vast number of non-genetic factors (e.g. parity, age, season, stage of lactation, etc.) that can influence cell count.

Factors That Affect Milk SCC.: The first factor influencing milk SCC is inherent animal to animal variation. What is of importance here is the level of SCC in milk of uninfected quarters. Reichmuth (129) reviewed studies showing differences in the average cell count of the milk of different breeds. The so-called highland breeds in Germany were 50,000 to 100,000 cells/ml lower in count than lowland breeds. Comparative studies face the difficulty that different breeds tend to be associated with different management practices. However, Reichmuth (129) concluded that susceptibility differences can be demonstrated both between and within breeds. In a more recent study (62) that compared two breeds of cattle differing in mastitis frequency, it was found that the breed with the higher infection incidence had the higher cell counts. Furthermore, these researchers found
Table 1. Types of cells in normal milk.*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (PMN)</td>
<td>0-11</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>10-27</td>
</tr>
<tr>
<td>Macrophages</td>
<td>66-68</td>
</tr>
<tr>
<td>Epithelial (ductal)</td>
<td>0-7</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>1</td>
</tr>
</tbody>
</table>

*Adapted from Lee et al. (84).
that the breed difference in cell count was equally apparent in 50 to 55 cows of each breed selected for freedom from mastitic infection during the current lactation. Finally, in another study (49) with Swedish cattle, researchers found that the most important source of variation in SCC was the individual cow, which accounted for 38-46% of total variation when all other effects were considered.

Coffey (32) summarized 13 studies in which heritabilities of cell count were estimated. Estimates ranged from zero to .77 with an average of about .24. He concluded that this average was twice the comparable results for clinical incidence and bacteriological tests. In a more recent review (156) Shook summarized heritabilities of the logarithm of SCC (LogSCC) from 5 studies with data sets having large numbers of commercial herds, and concluded that the heritability of LogSCC is lower than estimates reported in earlier studies. This review presented heritabilities of first lactation LogSCC ranging from .05 to .47 with a mean value of .17. Some research was based on one test per lactation and other studies used lactation averages. According to theory, $h^2$ should be higher for lactation average of several tests than for a single test (156). The author also concluded that these rather low heritabilities indicate that effective selection against (or for) LogSCC would require the use of pedigree and progeny information in addition to individual performance.

Also, if cows do differ inherently in their normal SCC, then genetic variation of two types must contribute to the observed heritability: variation due to SCC response to mastitis and variation in normal SCC. Thus, the magnitude of the genetic correlation between SCC and mastitis incidence is of paramount importance if a selection program to increase mastitis resistance based on SCC is to succeed. Observed correlations between the two variables have been inconclusive in defining their relationship. Young et al. (183) estimated genetic correlations of SCC with clinical frequency and bacteriological tests as .80 and 1.23 by paternal half sisters. Corresponding estimates by daughter-dam regression were .98 and .24. Finally, corresponding phenotypic correlations were .45 and .47. In a more recent study (32) Coffey found genetic correlations between measures of cell count and bacteriological culture results ranging from .133 to .472. He concluded that any selection program designed to reduce SCC should also reduce mastitis incidence.
If we were to use SCC as a parameter in breeding programs, then the relationship between this parameter and milk yield is obviously very important. Genetic correlation between milk yield and SCC may be somewhat lower than the correlation of yield with mastitis (156). Estimates have been obtained for a variety of breeds and lactation numbers in different populations. Averages of estimates within a study were .14, -.08, .46, .00, and .16 as reported by Kennedy, et al. (80), Ruvuna and Shook (136), Emanuelson and Philipsson (50), Coffey (32), and Monardes and Hayes (103). The tendency for positive genetic correlation indicates that sires' daughter groups with higher yield tend to have higher SCC. The positive genetic correlation between milk yield and SCC is in contrast to negative phenotypic correlations, typically -.10 to -.20 (156). The negative phenotypic correlation implies a tendency for cows with higher SCC to be lower milk producers. Presumably the negative association is mediated by mastitis which elevates SCC and reduces yield through damage to secretory tissue (156).

A second factor that affects milk SCC is presence or absence of infection. Related factors include species of pathogen, number of quarters infected, and sampling time. These factors can affect somatic cell count and distribution of counts as well. In a study by Bodoh et al. (18) Strep. agalactiae had the greatest impact on WMT as compared to C. bovis, S. aureus, S. epidermidis, and coliforms. Work by de Vries cited by Miller and Schultze (101) also indicated that streptococcal infections had the greatest impact on cell count.

A third factor which accounts for substantial variation in infection rates and SCC is the age of the cow. That average cell counts increase with increasing age of the cow, is accepted in the literature as indicating that the probability of being infected cumulatively increases with age (129). Several studies (99, 164, 178) have shown this relationship. It is also interesting to note a decrease in cell count averages in cows older than seven years-presumably resulting from selection (Zeidler et al. as cited by Reichmuth (129) ).

The stage of lactation at sampling time is a fourth factor influencing milk SCC. Between the first and third, or less frequently, the first and fifth days of lactation, there is a primary rise in cell count
(129). This increase of SCC in early lactation suggests that rates of new mastitis infections are highest in this phase of lactation (101). Throughout lactation there is generally a rise in cell count. The inflammatory responses become more severe throughout lactation and are exaggerated by the reduction in milk secretion as lactation advances (129). Other workers have also found this same tendency (3, 8, 17). However, in a more recent study (49), the authors found that lactation stage had little influence on test day LSCC when test day milk yield was included in the model. Their results indicated that the often reported increase in SCC towards the end of lactation is merely a 'dilution' effect. Finally, a rise in cell count just prior to drying off may well be due to a new infection (113). In conclusion, while there seems to be some correspondence of stage of lactation patterns for infection status and cell counts, there has not yet been a conclusive study about their true correlation, particularly in late lactation.

Season is a fifth factor causing variation in infection rate and possibly cell count. McDowell and McDaniel (90) reported that infection rate is highest for cows calving in summer. Bodoh et al. (17) found highest cell counts in summer and lowest in spring. Finally, a more recent study (133) found the lowest cell counts in milk samples obtained in spring and the highest counts in samples obtained in fall and winter. Of course, variation in climatic conditions among studies may have contributed to differences in results found by these studies.

A sixth category of factors that can affect mastitis frequency and cell counts, are those factors that contribute to the physiological state of the cow. Miller and Schultze (101) cited research that indicated high environmental temperatures led to increased SCC in milk without increasing mastitis incidence. Paape et al. (120) reported that transient increases in cell count were produced by using ACTH to mimic stress. The concentrations of milk somatic cells after cows were given injections of 0, 100, and 200 IU doses of ACTH were 19.7, 22.6, and 23.9 X 10⁶/ml, respectively. Physical stresses of the cow which cause an increase in SCC without inducing mastitis tend to reduce the correlation between SCC and mastitis incidence (101).
A seventh group of factors influencing mastitis incidence and SCC include various management factors. Thompson et al. (168) reported that variation in vacuum level of the milking machine is a contributing factor in mastitis. Also, Natzke (110) observed that herds with shorter machine-on times had less mastitis, while cell counts increased marginally in quarters that were overmilked. In addition, Carroll and Jasper (25) found that proper bedding management, keeping bedding clean and dry, was important in reducing mastitis, particularly coliform infections.

Finally, there are factors affecting SCC that have to do with technical aspects of determining cell counts in milk samples. These sources of variation include the method of cell count determination, diurnal changes, age of sample, and portion of milking sampled. Schultze et al. (147) published results of a study designed to compare the effectiveness of five screening test methods in determining milk cell count, using DMSCC as the standard. In this study, WMT and Milk Gel Index were the best tests based on cost-utility analysis. The precision of electronic mastitis detection equipment has been investigated by (57) and (68). Gebre-Egziabher (57) evaluated an electronic sensor as an instrument for early detection of mastitis. The conductivity ratio (conductivity of milk was continuously measured throughout the milking process and then a conductivity ratio was established) used in this method correctly identified 69% of the established cases of mastitis. Heeschen (68) reported a repeatability of .935 on SCC determinations made on duplicate samples and of .945 on repeated cell counts made on the same sample by Fossomatic cell counter. In another study (160) the authors presented limited data that SCC rise sharply at the end of milking, remain high for about 4 h after milking, dropped slightly in the next 4 h, and reached a minimum at milking time. With regard to the effect of milk sample age on cell count, one study (150) showed that SCC obtained by Fossomatic counters gradually declined up to 8 days of age. Also Mochrie (102) found that Fossomatic counts declined 6% in 10 days and up to 10% after 30 days. Finally, different portions of a milking vary in cell count. Cullen (38) found that there was no constant relation between foremilk and stripping but that strippings generally have higher counts.

Thus, in addition to genetic factors, a large number of environmental sources seem to be responsible for the observed variation in SCC. However, research (5, 6, 8) has shown that many of these
sources of variation can be accounted for by using herdmate comparisons and appropriate statistical procedures.

**Statistical Properties of the Optimum SCC Measure.** The various methods of determining somatic cell count in milk samples, express counts in the form: number of cells/ml. One important question is: what is the optimum function of SCC that best describes the health status of an animal's mammary gland? Shook (152, 153, 154) has performed considerable research with regard to the characteristics of the best measure of cell count. The frequency distribution of SCC in milk has positive skewness (i.e. the mean is greater than the median) and markedly heterogenous variance among groups (5). However, for the valid application of analysis of variance and related statistical procedures, data to be analyzed must have the following properties: 1) data for each group must be obtained randomly from a normal population; 2) the sampled populations must have equal variances (homogeneous variances); and 3) the effects of factors must be additive. Although not all data in nature, including SCC, meet these criteria, the desired characteristics may be approached by the proper transformation of the data. Researchers (6, 7, 45, 150) have investigated several transformations of SCC data, particularly log SCC and ln SCC. From his studies, Shook (155) has determined several statistical and biological characteristics that he feels the optimum cell count measure should possess.

First, the measure of cell count should have linear relationships with milk yield and probability of mammary infection. Recent research by Clabaugh (30) and Raubertas and Shook (128) has shown that milk yield declines linearly with increases in the natural log (ln) of SCC, while a curvilinear decline in milk yield is associated with SCC. In addition, research (157) indicates that ln SCC is a better predictor of the presence of pathogens in milk samples than SCC.

Second, the mean measure of the cell count measure should be near the median. Shook (155) found a correlation of .95 between the median SCC and the geometric mean of SCC (while only .78 between the median SCC and the arithmetic mean) and he suggested that the geometric mean or its equivalent mean of ln SCC should be better to use in analyses of SCC.
Third, the measure of cell count should be normally distributed. The normal distribution is desirable since only the mean and standard deviation would then be needed to characterize the frequency distribution, and tests of significance in analysis of variance require normality of residuals. Research (5) has shown that In SCC is the optimum transformation for achieving a normal distribution of cell count data.

Fourth, the variance among cows for the cell count measure should be uniform within subgroups such as herds and sires. Ali and Shook (5) computed within herd variances among cows for 52 herds. Bartlett's $X^2$ test for heterogeneity of variance was 399 ($p < .01$) and highly significant for SCC. A logarithmic transformation of the data, In SCC, decreased the $X^2$ for heterogeneity of variance to 39 and nonsignificance. The data suggests that the subclass variances for cows within herds were equal for In SCC.

Fifth, the measure of cell count should lend itself to be expressed accurately on a lactation basis. It has been found (7) that 65% of total variation in In SCC is due to variance among tests within lactation, which illustrates the large influence temporary environmental effects have on cell count. The averaging of several tests over a lactation reduces the temporary effects and gives a better measure of the cow's true level (152). Shook (152) has also found that lactation average In SCC was better than lactation average SCC in predicting the presence of bacterial pathogens and milk yield loss. In addition it was found (7) that the $h^2$ of lactation average In SCC is greater than for lactation average SCC (.79 vs .71 estimated from one model and .73 vs .56 estimated from another model).

Finally, the cell count measure should accurately summarize cell counts of cows within a herd. The objectives of a herd summary is to measure differences in herds and changes over time within herds. Research by Shook et al. (158) indicates that the lactation average In SCC is preferred to lactation average SCC in summarizing herd cell count levels, because the former is less susceptible to erratic jumps in cell count for a few cows in small herds. These jumps without reflecting important changes in herd performance, would definitely affect the herd average SCC (152).
The only difficulty of ln SCC is problems dairymen may have in relating a logarithmic sire evaluation to somatic cell count reported for individual cows. This difficulty may be avoided if ln SCC is expressed as a linear score (LS). The LS suggested by Shook (155) and which has been adopted by National DHI as the measure of choice to express monthly DHI test-day cell counts in the U.S.A., is a base 2 log transformation of SCC. It also is a linear transformation of the natural log of SCC and has the same properties as the ln SCC. The new LS ranges from 0 to 9, and each increase in SCC score corresponds to a nearly constant decrease in milk yield.

Selecting for High or Low Milk SCC?: In recent years there has been a debate with regard to the appropriate direction of selection on SCC, if such a selection for increasing genetic resistance to mastitis based on SCC should be followed at all. Associated with this debate is the confusion between the extent to which SCC is a defense mechanism and the extent to which it reflects the current presence or absence of mastitis. Some of the studies that led to this debate will be mentioned briefly.

Paape et al. (124) have reviewed results which establish certain fractions of SCC (PMN and others) as a principal defence mechanism against udder infection. In a study by Bramley and Neave (22) it was found that cows were less susceptible to artificial infection when SCC in the mammary gland was high from a previous infection. In another study, Paape et al. (122) demonstrated that when an irritating device (plastic coil) was placed in the mammary gland, the cow became less susceptible to artificial infection due to the elevated PMN in milk. The protective role of PMN in the lactating udder was also shown by Jain et al. (72), who produced neutropenia in cows by injecting them with equine anti-bovine leukocyte serum. When udders were then challenged experimentally with Aerobacter aerogenes, the neutropenia led to unrestricted multiplication of the coliform within the infected quarters. In another study, Schalm et al. (137) reported that quarters with high cell counts were more difficult to infect experimentally than those with low counts. Linde et al. (87) in a more recent study has found this same effect. In another study (139) Schalm et al. reported that udders of cows chronically infected with Staph. aureus became gangrenous when neutropenia was
produced with equine anti-bovine leukocyte serum. Other work by Poutrel and Lerondelle (126) indicated that quarters with less than 500,000 cells/ml could be artificially infected by Staphylococci with an 82% certainty, in contrast to quarters exceeding 500,000 cells/ml (67%). Such results suggest that selection for reduced SCC may lessen the cow's ability to respond to infection and that some minimum SCC (e.g., 200,000 to 500,000 cells/ml) may be most desired. It has, in fact, been suggested that selection for decreased SCC might yield a reduction in mastitis incidence in early generations of selection, but later produce an increase in mastitis problems by reducing the abilities of cows to respond to bacterial infection (22). Similar conclusions have been also reached by Miller and Schultze (101) in their extensive review.

However, Jones et al. (77) reported somewhat conflicting results, based on a study with 67,707 milk samples from cows in 30 herds over a 4-year period. Specifically, these workers found that: 1) a high proportion of milk samples exhibit low SCC (45% < 100,000; 24% < 50,000), 2) samples with low SCC have low infection rates as determined by bacteriological culture (62% negative; 94% free of major pathogens), and 3) the greatest rate of decline in milk yield with increasing SCC is at low SCC. These results suggest that even low SCC are indicative of infection or stress and have detrimental effects on milk yield. In another study Coffey et al. (33) found cows with low SCC in initial tests of first lactation were no more at risk to infection later in first lactation, or in subsequent lactations, than were cows with average and high SCC. This does not support the notion that cows with low SCC lack the ability to respond to bacterial infection. Coffey (32) also found genetic correlations among measures of cell count and bacteriological culture in the range of .133 to .472 and he concluded that any selection program designed to reduce SCC should also reduce mastitis incidence. In addition, it has been accepted that milk SCC of less than 500,000 are normal (143). However, the threshold of 500,000 cells/ml has been questioned. Reports demonstrate loss of milk yield at counts of less than 100,000 cells/ml (128). Perhaps parenchymal damage is associated with milk cell concentrations (< 500,000 cells/ml) considered to be well within the range of normal milk (55). In one recent study (55) dealing with a data set in which more than half the quarter samples had SCC of less than 200,000 cells/ml, the authors reported that with every doubling of cell count
there was a loss of .12 kg milk in comparisons within cow between udder halves. Results like these do not support the notion of selecting for high SCC. Finally, two studies by Grootenhuis (61, 63) also show the tendency of some researchers to believe that selection for low SCC would be desired. In the first study, Grootenhuis (61) showed that paternal half-sib groups with high SCC in first lactation had higher susceptibility to mastitis in later lactations. One daughter group with high SCC in first lactation was more susceptible to experimental mastitis in third lactation than another group with low SCC. In the second study the author found that two progeny groups with high SCC in first lactation had significantly higher SCC and higher rate of clinical mastitis in later lactations when compared with two other progeny groups whose SCC was low in both first and later lactations.

Overall, to answer the question of whether one should select for high or low milk SCC most researchers agree that more investigation is needed. It has been suggested that areas that need to be more investigated should include the actual role of PMN leukocytes in phagocytosis, and the effects that selection for low SCC in dairy bulls might have later in the health status of these bulls' daughters. Current genetic correlations between SCC and mastitis on one hand, and SCC and milk yield on the other have been derived from static populations. However, to answer questions like: does natural variation in cows' SCC represent differences in their ability to respond to mammary infections or does it represent differences in the cows' abilities to preclude organisms from the udder? we feel like it is only through selection that an answer could be derived.

**Induced Diapedesis of Leukocytes from Blood to Milk**

It has been mentioned earlier that SCC in milk increases dramatically after an infection has been established in the mammary gland. Also, this increase in leukocyte numbers in the mammary gland is one of the most important responses of the organism against bacterial infections. Recently, studies have being conducted to investigate the efficacy of using an abraded intramammary device
(AIMD) to increase the milk SCC and thereby reduce mastitis incidence. In a review paper (123), Paape et al., summarized results from 13 studies designed to study the effect of intramammary devices (IMD) on mastitis incidence and milk production. The authors concluded that: 1) the concentration of milk SCC in stripping milk needed to prevent establishment of intramammary infection (IMI) appears to be ≥ 900,000 cells/ml; 2) an improvement that was made to the originally used IMD (abrading its surface) increased milk SCC in stripping milk to concentrations that provided 60% protection against establishment of infection after challenge with Strep. uberis or E. coli; 3) the milk SCC in IMD quarters infected with minor pathogens was 2 to 4 times greater than quarters that were infected but contained no IMD or quarters that were not infected but contained an IMD; and 4) the effect of IMD on milk production is not clear but there seems to be a tendency towards less milk being produced by IMD quarters when compared to non-IMD quarters. Furthermore, in a recent study with 3,660 Israeli dairy cows (185), it was found that there were only 164 cases of clinical mastitis among IMD cows and 366 cases among controls. Microorganisms responsible for the infections were Staph. aureus and coliforms.

In addition, there are many studies in which SCC in milk have been caused to migrate in large numbers from blood to milk and through the secretory tissue of the mammary gland into the alveolus of the gland by using chemical stimulants such as Eschericia coli endotoxin (140, 148, 167), oyster glycogen (4, 121), or even physiological saline (121). Endotoxin, or lipopolysaccharide (LPS) is a heat-stable toxin found primarily in Gram-negative bacteria. It is a potent pyrogen that causes increased vascular permeability, and release of endogenous pyrogens from leukocytes with an elevation in body temperature (14). Schultze and Paape (146) noted that doses of .1 mg were sufficient to mimic the early effects of acute coliform mastitis and that the induced increase in SCC returned to pre-infusion levels after three days. In a recent study (167) the relationship between SCC and milk yield was studied to determine if leukocyte migration into the udder affected mechanisms of milk synthesis and secretion. Six lactating Jersey cows were used and separated into two groups of three animals. The first group was given intramammary infusions of 10 µg endotoxin per quarter every three days, while the second group served as a control. After 14 days, the groups
were switched and treatment was repeated. Endotoxin treatments significantly increased SCC with means of 4,970,000 cells/ml for treated cows and 725,000 cells/ml for controls. In addition, treated quarters often produced clotted milk even though the quarters were not infected. Milk yield was significantly lower in treatment (mean = 6.9 lb/milking) vs control (mean = 8.5 lb/milking) cows (P < .01). It was also stated that udders never appeared edematous in contrast to observations by Schalm et al. (137) who found that doses of 20 μg consistently produced maximal swelling. In the same study (167) it was reported that SCC decreased sequentially from an average of 9,255,000 cells/ml (9 and 24 h after infusion) to 1,486,000 cells/ml (72 h after infusion). Highest SCC concentration was observed 24 h after infusion. An important conclusion in this study was that as leukocyte counts increased, milk production decreased; not as a result of bacterial infection, but presumably from migration of leukocytes through secretory epithelium. However, this was shown to be a reversible process. By allowing leukocytes to decrease, milk yield increased, thus rejecting the idea that diapedesis of leukocytes through mammary tissue causes permanent damage to epithelial cells. Findings similar to these were also noted by Schalm and Ziv-Silberman (140), and support the theory that endotoxin may be a nonspecific stimulator of the immune system (14). In another study (145) six cows in their first lactation received 1μg of E. coli endotoxin intracisternally once a week after a morning milking for 20 successive weeks. Mean response to endotoxin varied between 5,790,000 and 13,400,000 cells/ml among quarters. Also, Todhunter et al. (169) gave intramammary infusions of 25 μg endotoxin to four Holstein cows at approximately 45 days of lactation. The authors observed a maximum SCC of 7.5 (log10) (or 31,622,776 cells/ml) at 10 h post-infusion. Milk SCC returned to preinfusion values by 120 h. In a study by Gupta et al. (64) normal leukocyte counts in milk of guinea pigs were between 10,000 and 6,000,000 cells/ml. However, guinea pig milk SCC rose to 45,000,000 cells/ml after intramammary infusion with 500 μg Salmonella typhi endotoxin. This increase was observed at 6 to 8 h after infusion (91). In addition, these same authors (91) injected 500 μg/.5 ml of E. coli endotoxin via the streak canal of the mammary gland of guinea pigs. They observed that the highest number of PMN leukocytes at 8 h after infusion were found: 1) between epithelial cells and 2) within the lumen of the mammary gland. By 12 h, numbers had decreased from 8 h. Finally, in another study (92) with guinea pigs,
the authors tried to determine 1) the minimal concentration of E. coli endotoxin that would stimulate leukocytosis and 2) the concentration of E. coli endotoxin that would produce maximal leukocytosis in the mammary gland of the guinea pig. Endotoxin concentrations of .005, .05, .5, 5.0, and 50.0 μg/ml in 1 ml were used to stimulate migration of PMN leukocytes in milk, observed at five times (2, 4, 6, 8, and 12 h) postinjection. There were three animals in each time by endotoxin concentration group. Each animal served as its own control by having sterile saline injected into one mammary gland and endotoxin into the other. A control group of three animals were not injected with saline or endotoxin. Leukocyte numbers in the milk of uninjected mammary gland did not differ from those in milk of saline injected glands. The difference in mean SCC between saline injected and .005 μg/ml endotoxin injected glands was not significant (P > .05) indicating that minimal concentration of E. coli endotoxin necessary to stimulate leukocytosis was greater than .005 μg/ml. Also, there was no difference between mean numbers recruited by the 5.0 and 50.0 μg/ml endotoxin doses at 4, 6, 8, and 12 h. The chemotactic effect and the rate of PMN leukocyte recruitment was maximal at the 5 μg/ml endotoxin dose. The effect of time on PMN cell recruitment was highly significant (P < .0001), with the highest number of SCC for all doses (except that of 50 μg/ml) observed between 6 and 8 h postinjection. For all doses (except that of 50 μg/ml) SCC decreased at 12 h after injection. There was also a significant interaction of dose x time (P < .05). Results of this experiment are shown in Table 2. From these results many comparisons between mean SCC for different doses and times can be made. However, to our knowledge, no study has used the technique of induced leukocytosis to study differences between individuals with regard to their ability to respond to chemotaxis challenge.

Mammary Gland Infections in the Laboratory Mouse

Problems of husbandry, including management and costs, have always caused difficulties in the use of cows for the study of bovine mastitis. Goats and sheep have been used as a substitute, but with these species husbandry problems are also considerable (29). The laboratory mouse provides an
Table 2. Average SCC (log_{10} and SE) recruited per 1 ml of milk with 5 doses of E. coli endotoxin (µg/ml) at 5 times into the mammary gland of guinea pigs

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1. Adapted from McKenzie, W.N., and R.R. Anderson (92).
2. X = mean SCC (log_{10}).
3. n = number of animals.
animal model for studying mastitis that is inexpensive and readily maintained by laboratories with standard facilities. This model has been used with success in investigating intramammary infections. Chandler (29) used the BSVS strain of mice for intramammary inoculations with bacteria commonly associated with naturally occurring bovine mastitis, and observed macroscopic and histological responses in the mice involved. Female mice that had produced their first litter and were at the 10th-15th day of lactation were used in this experiment. Offspring were removed from the lactating mice and disposed of about 1-2 hr before inoculation. Bacteria inoculated included: Staph. aureus (strain S57); Strep. agalactiae (strains S13 and 122); Coryn. pyogens (strain PM1); Escher. coli (strain GS1); and Pseud. aeruginosa (strain GS2), all of which are commonly isolated from cases of bovine mastitis. The mice were lightly anesthetized with ether and a Hamilton 33G steel needle was used to deliver the inoculum through the streak canal of the mammary gland and at a position of about two-thirds of the length of the teat. The author noted that in a high proportion of cases the needle penetrated at a point other than the streak canal orifice, but he stated that this did not appear to alter the effect of the procedure. The results of inoculation of dyes and observations on the production of mastitis indicated that each mammary gland was anatomically and functionally distinct from its neighbor in the same manner as in the bovine species. The mouse, like the cow, has one teat duct or streak canal per teat. For convenience of recording, the teats were designated as follows: left side, from anterior to posterior, L1, L2, L3 (thoracic group) and L4, L5 (abdominal group); right side, similarly R1, R2, R3 and R4, R5. The abdominal mammary glands were larger than the thoracic ones. Usually, active material was inoculated into teats on the left side (L3 and L4) and control material into the corresponding teats on the right side of other mice of the group. Examinations performed on inoculated mice included clinical observations, post-mortem examination, histological examination and bacteriological counts. These examinations revealed that BSVS mice were, in general, very susceptible even to small numbers of the Staph. aureus strain (S57). The lowest titre applied was equivalent to 3.5 viable units per inoculated teat. No mastitis was observed in the neighboring mammary glands. In addition, experiments with Streptococci indicated that the degree of susceptibility to infection with this organism was more variable and lower than that experienced with Staphylococci. This has its parallel in the bovine
species where experimental infection with Streptococci by the intramammary route also produces variable results (71). Finally, experiments with C. pyogenes, Ps. aeruginosa and E. coli also indicated that mastitis could be produced in mice by intramammary inoculation of these species. The character of histological changes produced by different bacterial species in the mouse resembled those produced by the same species in cattle (78). These changes included enlarged glands, reddish brown in colour and edematous, swelling of the acinar cells and finally necrosis of the mammary tissue. Chandler concluded that the size of the mouse mammary gland was very convenient for histological and other studies requiring examination of the whole gland. He also noted that experimental mastitis in the mouse provides a useful model for certain fundamental studies and for experiments, such as those of a screening nature, prior to studies on cattle.

In a more recent study Anderson (10) compared the effect of cell-free toxic preparations and live Staphylococci derived from two Staphylococcus strains. Strain BB was known to be of high virulence and strain Mexi of low virulence in mice and cattle (9, and Reiter et al., 1971, as cited by Anderson (10)). Both strains, originally isolated from clinical cases of mastitis in cattle, were coagulase positive and produced α- and β-haemolysins on 5% (v/v) ox-blood agar. Lactating mice of the BSVS strain between the 3rd and 6th day of lactation were used. Offspring were removed from their mother 1 hour before inoculations were given. Both Staphylococci cultures as well as free-cell Staphylococci toxin were injected in the mammary glands of the mice involved. Mice were anesthetized with ether and .1 ml of the appropriate strain of Staphylococcus or toxin preparation or appropriate control material was inoculated into the glands R4 and L4. Toxin preparations of the two strains showed to be similar in their in-vitro properties and in their clinical and histopathological effect when inoculated into the mammary gland of mice. However, when live Staphylococci were inoculated, a difference in virulence corresponding to that seen in cattle (high for BB and low for Mexi) was detected by the clinical response, and by the histopathological appearance, the number of organisms, and the amount of α-lysin present in the infected glands. The mouse again proved to be a useful animal model to study intramammary infections.
Peripheral Leukocytes in Mice: Selection Results

It has been stated earlier that there are results from experiments with laboratory mice suggesting possible genetic control over functions and numbers of blood leukocytes. In an early study Gowen (60) found that strains of mice resistant to Salmonella typhimurium had an intracellular digestive enzyme in macrophages that was absent in susceptible strains. Meyer et al., (93) practiced two-way selection of mice for phagocytic ability of their peripheral leukocytes. They found realized heritabilities of .30 for high ability, and .25 for low ability. In a more recent study Verghese et al. (173) compared two strains of mice which differed genetically in susceptibility to bacterial endotoxin. The mutant mouse strain C3HeB/FeJ is known to be susceptible to the inflammatory and/or lethal effects of E. coli endotoxin whereas the strain C3H/HeJ is resistant (173). Susceptible mice had a 50% decrease in circulating leukocytes 3 hours after dose, whereas resistant mice required a 100 times greater dose to achieve a comparable decrease in leukocytes. Thus, peripheral leukopenia produced by endotoxin was a very sensitive discriminator between the two strains. However, Weir and Schlager (176) found somewhat conflicting results. The objective of their investigation was to produce high and low leukocyte lines of mice to study the contribution of leukocyte number to natural resistance level. They selected two strains of mice for high (LCH) and low (LCL) leukocyte counts. At the same time they maintained the unselected control line T, which was the result of random mating of a crossed population. Total leukocyte counts as well as differential counts were made from blood samples by standard laboratory procedures, when the mice were 30 days of age. Selection for high and low total counts was practiced for 20 generations. Means and standard errors of 25 generations of the two selected lines are given in Table 3 along with data from line T at generation 0 and corresponding to selected generations 2, 5, 7, 10, and 16. The procedures and the results of their experiment are summarized as follows:

1. The mean of total leukocyte counts from 118 outbred mice (T strain) was 9121 cells/mm³ (log count 3.960±.0128).
2. Selection was based on family and individual merit in a closed system and matings were brother by sister.

3. By generation 13, the maximum difference between strains was achieved with arithmetic and log counts as follows: LCH 17,500 (4.244 ± .0162); LCL 4,000 (3.602 ± .0318).

4. Although the selection differential was uniform in the two lines, the intensity of response was greater in LCH. Response in the LCL was more gradual, but continued until the end of the experiment. However, the outbred T mice also followed a downward trend, indicating environmental influences.

5. Heritability estimates by four methods were: 22.4, 20.3, 18.7, and 17.4 percent.

6. The major portion of the difference in leukocyte count between LCH and LCL was accounted for by lymphocytes, although LCH mice had more cells of all categories. In LCL mice the number of PMN and eosinophils in blood increased under downward selection pressure for total count.

7. The selected lines did not differ significantly in numbers of progeny or weaning weight.

8. There were no significant differences between strains in resistance to a standard dose of live organisms of S. typhimurium, the toxic effects of massive doses of heat-killed organisms, or to reinoculation with live organisms after immunization, thus rejecting the hypothesis that increased peripheral leukocyte number per se enhances resistance.

9. After an acute dose of 650r of X rays, survival were: LCH 24/30 (80 percent); LCL 9/25 (36 percent).

Considerable research in the field of circulating leukocytes in mice has also been undertaken by Chai and coworkers. In an early study Chai (27), compared two strains of mice, which originally had been selected for large and small body size, with regard to their blood leukocyte counts. White blood cells averaged 8380 in the large strain and 2320 cells/mm³ in the small strain. For a comparison, in ordinary inbred strains of mice leukocyte counts range from 6,000 to 11,000 cells/mm³ of blood (135). Also, in the study by Chai (27), neutrophils and eosinophils in the small strain were more severely reduced as compared to lymphocytes and monocytes. By using hybrid strains F1 and
Table 3. Total leukocyte counts of LCH, LCL and T mice.  

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1. Adapted from Weir, J.A. and G. Schlager (176).
2. Mean leukocyte number in cells/mm$^3$ of blood.
3. $X = \text{mean (Log leukocyte number)}$. 

57
F2 (hybrids of the small and large strains) he estimated that the genetic contribution to the variation in counts, in F2, was 50%. Chai concluded that leukopenia in mice is an inherited character.

In another study Chai (28), applied directional selection for leukocyte counts starting from a hybrid mouse stock. This hybrid stock was derived by crossing six inbred strains. One of these strains had a high leukocyte count, another strain was leukopenic and the remaining strains were intermediate. Two lines were formed using directional selection based on individual merit for high (HLC) and low (LLC) total leukocyte counts. In addition, a random-bred line derived from the same hybrid stock was maintained and designated RLC. Single matings were set by random pairing, but sib-mating was avoided. Blood samples were obtained from the lateral tail vein by incision, when the mice were 60 to 90 days old. Samples were taken for total and differential counts. No anticoagulants were used, and the total counts were made instantly. As far as differential counts, four types of cells were recorded: neutrophils; eosinophils; lymphocytes; and monocytes. Eleven generations of selection for total leukocyte counts produced two lines of mice which differed not only in total cell counts but also in differential counts, reproductive performance, resistance to irradiation, and body weight. Means and standard errors of total and differential counts for the early (0-2) and and late (9-11) generations of selection are given in Table 4. Responses to selection for high and low counts were asymmetrical. In the first two generations, responses were irregular; thereafter they were large in the low line (LLC) for two or three generations and then became small in comparison with those of the high line (HLC). At eleven generations of selection, the mean leukocyte count of HLC was about three times that of LLC. Responses of the different cell types were proportional to their individual percentages of the total counts. The percentages of the individual cells during the course of selection showed the following pattern: The percentages of the two major cell types lymphocytes and neutrophils, apparently influenced by selection, showed significant changes. In the first three generations no noticeable differences occurred between the three lines; the levels of percentage were rather high for lymphocytes and low for neutrophils, with a general tendency toward gradual decrease in the percentage of lymphocytes, and increase in that of neutrophils through the third or fourth generation. Thereafter, the three lines separated. The de-
Table 4. Means and SE of total leukocyte counts and differential leukocyte percentages for the early (0-2) and late (9-11) generations of each line.1

<table>
<thead>
<tr>
<th>Generation</th>
<th>Line</th>
<th>n³</th>
<th>Total²</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<td>X² SE</td>
<td>X SE</td>
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<td>549</td>
<td>7056</td>
<td>2630</td>
<td>12.01</td>
<td>1.62</td>
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<td>666</td>
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<td>1.46</td>
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<td>14.08</td>
<td>1.46</td>
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<td>13.31</td>
<td>2.46</td>
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<tr>
<td></td>
<td>LLC</td>
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<td>5316</td>
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<td>2.91</td>
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<td>1446</td>
<td>18.15</td>
<td>2.91</td>
<td>77.19</td>
</tr>
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</table>

1. Data revised from Chai (28).
2. Total leukocytes/mm³ of blood.
3. n = number of mice (females only).
4. X = mean of total leukocyte counts or differential leukocyte percentages
5. Line of high leukocytic count.
6. Control line.
7. Line of low leukocytic count.
crease in lymphocyte percentage and increase in neutrophil percentage continued in the LLC line, whereas in the HLC line the reverse became true. Thus the divergence between the two selected lines with respect to the percentage of these two cell types widened with the advance of generations. The percentage levels for both cell types remained intermediate in the RLC line. Finally, the two minor cell types, eosinophils and monocytes, showed larger percentage variations within than between lines, perhaps because of sampling errors due to their scarcity (28). However, more generations showed higher percentages of both eosinophils and monocytes in the LLC than in the HLC line. Heritability estimates based on selection differential and response as well as on sib relationships yielded values ranging from .15 to .39. In addition, beginning with the third generation, increased variation in body weight occurred in the LLC line, indicating decreased fitness. Additional indications of such a decrease were a sudden drop in reproductive performance, with a high percentage of sterility in the sixth generation, and greater mortality from X-irradiation in LLC mice than in those of the other two lines. Up to the seventh generation the increment of inbreeding in the LLC line was about the same as that in the HLC, suggesting that low counts may be the main factor causing decrease in fitness in the early generations. From the eighth generation LLC mice were further hampered by a rapid increase in rate of inbreeding—an additional factor affecting fitness.

Finally, in a more recent study Silver and Chai (159), examined the immune response to sheep red blood cells (SRBC) of the two previously formed lines HLC and LLC. Low mice LLC responded to multiple injections of $4 \times 10^7$ SRBC with predominant IgM (the most important isotype promoting phagocytosis) response whereas high mice HLC produced IgG almost exclusively, even after a single injection of SRBC. F1 mice responded like the LLC parent and backcross data implied genetic control at multiple loci.
Milk Production in Laboratory Mice

Several methods of determining daily or lactational milk yield in mice have been investigated. In an early study Falconer (52), suggested that 12-day litter weight, expressed as a percentage of the mean litter weight might provide a useful measure of milk yield in mice. Twelve days after birth might be viewed as the time of maximum maternal influence since the young open their eyes at 13 to 14 days of age and begin to become less dependent on the mother for nutrition. Since first theorized, several studies have been initiated to test the hypothesis proposed by Falconer. Bateman (11), through the use of cross-fostering experiments found in 160 young suckled in 20 litters, that only 32% of the total variance in 12-day litter weight was due to postnatal origin, though 73% of this variation was maternal. The results suggested that 12-day litter weight was not an accurate measure of milk yield. The debate on whether 12-day litter weight is an accurate measure of milk yield in mice continued and more recent studies have been published on the subject. Various studies (36, 118, 182) have shown that variation in 12-day litter weight was accounted for by postnatal maternal effects in proportions 71.5%, 70-80%, and 80% respectively. In addition Eisen et al. (48), found that postnatal maternal influence explained 56% of the variance in 12-day individual weight, 11% of which resulted from postnatal maternal genetic effects. Finally, Robinson (134), in an experiment designed to study in part the relative magnitudes of direct additive genetic, maternal additive genetic and direct-maternal additive covariance for individual 12-day body weight, found that the direct additive genetic variance accounted for 40.5% of the total phenotypic variance while the maternal additive genetic variance accounted for only 8.2%. Also, there existed a small negative covariance between the direct and maternal additive genetic variances. The postnatal maternal environment accounted for 54.4% of the phenotypic variance. Based on the above evidence, 12-day litter weight is probably a useful phenotypic measure of postnatal maternal ability.

Hanrahan and Eisen (66) determined the lactation curve in mice by direct mechanical milking procedures. At day of birth (day 0), the litters were assigned at random to a particular day and then the dams were milked on that day only. Each litter was used only once. One injection of oxytocin,
0.5 units, was applied before milking and another ten minutes later. The milking was done in two rounds. The lactation curve reached a maximum (approximately 1.8 g/day) between day 12 and 13 postpartum. The curve was significantly quadratic ($P < .001$) and accounted for 41% of the observed variation in milk yield. Enzeman (51) reported the same quadratic shape of the lactation curve in mice, but the peak occurred at about day 10 postpartum.

Bateman (12), proposed that the litter itself be used to estimate milk production. He found that the removal of the litter from the dam for periods of 6 h and 11.5 h before suckling and weighing the entire litter at the same stage of its digestive process (either after long periods of starvation, when the alimentary tract could be expected to be almost empty; or at the end of suckling sessions, when it should be full) would yield adequate measure of milk yield. He concluded that the output of the gland was not affected by the dam being separated from her young for as long as 6 h. It seems that engorgement of the glands will not seriously inhibit production until the dam has been separated from her young for about 10 h.

Jara-Almonte and White (75), used the technique proposed by Bateman (12), to characterize the shape of the lactation curve and to estimate milk yield in 635 laboratory mice. The authors tested three different isolation periods (of the young). The three periods were 4 h, 6 h, and 8 h separation. These periods were compared for the effect on body weight of the young and on amount of milk. Although the means indicated a decrease in 21-day litter weight and individual body weight for the three isolation periods when contrasted with the continuous suckling control, individual comparisons among the three isolation periods were not significantly different ($P > .05$). Milk yield per female was similar for 6 and 8 h of isolation. Isolation of litters for 4 h resulted in reduced measures of milk yield ($P < .01$). Thus, it was established that 6 h removal was optimal for estimating milk yield. After 6 h isolation, the young were allowed to suckle for 1.5 h and the difference between pre-suckling and post-suckling litter weight was recorded as the daily milk yield. Daily measures of milk production in the dam were recorded from day 6 through day 21 of lactation. From regression of average daily milk yield on day of lactation second and third degree polynomial equations described lactation curves which accounted for 92.3 and 98.6% of observed variation in milk yield.
milk yield. These percentages were considerably larger than the 41% reported by Hanrahan and Eisen (66) for the lactation curve described by mechanical milking procedures. Peaks of the two lactation curves described by Jara-Almonte and White (75), occurred at days 13 and 14 for the second and third degree polynomial. The periods of peak production compared favorably with the study by Hanrahan and Eisen (66). However, the maximal yield per dam per day (3.1 g) in this study (75), was higher than the corresponding yield of 1.8 g in (66). In addition, multiple regression procedures were used to develop prediction equations to estimate total milk yield from observations on various lactation days. Different combinations of daily records to estimate total milk yield, regression coefficients, corresponding standard errors, and $R^2$'s are in Table 5. The regression coefficients in Table 5 are those for the particular combination of daily yields which resulted in maximum $R^2$'s. Measurement of milk yield on days 8, 10, 13, 15, 18, and 20 provided excellent prediction ($R^2 = .86$) of lactation milk yield.

Munford (105) studied the development of the mouse mammary gland by histometric and biochemical techniques, and showed that the activity in the mammary gland reaches a maximum between day 10 and 13 postpartum. The total glandular tissue (mm²) was a maximum about day 12 and the proportion of glandular tissue reached a peak about day 11 or 12. The number of nuclei per alveolar section as well as the number of nuclei per histological section were a maximum at about day 10. Munford (106) showed in mice that DNA content of the glands was greatest at approximately day 11 postpartum. Cole (35) found that the glandular tissue in mice reached a maximum at day 12 of lactation. Therefore, the shape of the lactation curve, and the day of maximum yield, reported in previous studies, generally, appear to be in agreement with the results reported by Munford (105, 106) and Cole (35).

No estimates of the heritability of milk yield in mice are available in the literature. However, heritability estimates for 12-day litter weight which has been utilized as an indicator of lactational milk yield, have been estimated. Miller et al. (97), Vinson (174), Eisen et al. (48), Young et al. (182), and Cox et al. (36) reported values of 0.08, 0.02±.18, 0.11±.02, 0.40 and 0.31 respectively.
Table 5. Regression coefficients and $R^2$ values for the prediction of total lactational milk yield** based upon production for varying numbers of days in the lactation periods*.

<table>
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<th>Number of days</th>
<th>Regression coefficients and standard errors</th>
<th>$R^2$***.</th>
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<tr>
<td>13</td>
<td>$3.927 \pm .21$</td>
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<td>$3.388 \pm .20$</td>
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</tr>
<tr>
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<td>-.464 \pm .11</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>4</td>
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<td>-.198 \pm .12</td>
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<tr>
<td>13</td>
<td>$2.951 \pm .16$</td>
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<tr>
<td>5</td>
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<td>.083 \pm .11</td>
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<td>13</td>
<td>$2.222 \pm .13$</td>
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<tr>
<td>15</td>
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<tr>
<td>18</td>
<td>$1.802 \pm .10$</td>
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<tr>
<td>20</td>
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</table>

*Adapted from Jara-Almonte, M and J.M. White (75).

**Mean lactation milk yield was 38.01 \pm .27 g for the 635 females represented.

***Values are those for the particular combination of days within each number of days group which resulted in maximal $R^2$ values.
Materials and Methods

Source and Management of Mice.

The animals used in this investigation were drawn from a laboratory stock of the panmictic strain of I.C.R. albino mice originally obtained from the Institute of Cancer Research, Philadelphia. Three reproductively distinct lines were randomly assigned from this stock maintained currently in the genetics mouse laboratory of the Department of Dairy Science, Virginia Tech, Blacksburg, Virginia. Fifty litters were assigned to line LSCC (low somatic cell counts), fifty litters were assigned to line HSCC (high somatic cell counts), and thirty-six litters were assigned to a biological control line CSCC. The purpose of keeping a control line was to account for environmental changes over time (23) in the process of selection on SCC.

Animals were maintained in opaque polypropylene cages approximately 27 cm x 17 cm x 13 cm, with metal cage tops which served as feed hoppers and held water bottles. A natural cellulose bedding material derived from clean corn cobs1 was used, which was substituted with clean material

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1 Andersons Cob Division, Maumee, Ohio
weekly. A high energy feedstuff was fed to mice from the time of mating until weaning. This feedstuff contained crude protein 17%, crude fat 11%, crude fiber 3%, ash 6.5%, minerals 2.5% and a full compliment of vitamins. From weaning to next mating mice were fed a lower energy formula pellets. The formula contained protein 22%, fat 5%, fiber 5%, ash 6% and a full compliment of vitamins and minerals. Water and food were both available to mice, ad libitum at all times except for the time of determining milk yield as will be explained later. The temperature in the laboratory was maintained at 22° ± 2°C, and the relative humidity at 50 to 60%. In addition, a 1:1 light to dark ratio was constantly kept in the room. Thus, the three lines were housed together and managed identically (44).

Laboratory Procedures

**GENERAL**

Selected lines (LSCC and HSCC) were perpetuated by 50 single pair matings each generation. The control line, CSCC, was perpetuated by 36 single pair matings. Matings within a line were at random, except that full-sib matings were avoided in all lines. Only virgin males and females 6 to 9 weeks old were used. In addition to these regular matings, "replacement" matings were also available in each of the three lines. These replacement matings were used only as needed to substitute for any regular matings which could not be made due to the death of one or both of the mice involved in a regular mating. To distribute work loads, each generation was mated in three mating groups, one week apart, with one-third of the matings in each line made in each mating group. At 10 days after mating, males were removed from the mating cages and were killed. The females were placed in individual maternity cages. Starting from day 18 after mating the females were checked daily for littering. At littering, the number born alive, sire and dam number, line number,

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2 Mouse Chow #5015 from Ralston Purina Comp., St. Louis, Missouri

3 Agway prolab rat/mouse/hamster 3000, from Agway Inc., Country Foods Division, Syracuse, NY
litter number, birth date and mating group were recorded. The first of the four digits of the dam identification number denoted the generation number. Where possible, litters in all lines were standardized at birth to 5 males and 5 females by removing excess pups and adding foster pups to litters of less than 10. Fostered young were from excess pups in the same line, born on the same day, and were tail-clipped and discarded at weaning (21 days). Litters of size less than six and those which could not be augmented to at least eight pups were recorded and discarded. The standardization of the litters at birth was done to stimulate maximum milk flow in the dam (12). Non-fostered mice were individually identified by toe notching and sexed at 12-days of age. Their identification number, sex code number and number of mice at day-12 were also recorded. Dams of litters were discarded at day 21 of lactation.

Several tasks had to be performed daily on a fairly large number of mice. The various duties to carry out on specific lactation days of the female mice were:

- Standardization of litters to 5 males and 5 females and tail clipping of the fostered mice at day of birth (day 0).
- Collection of milk samples and determination of SCC from all lactating females at days 9, 14 and 18.
- Determination of milk yield from all lactating females at days 8, 13, 17 and 20.
- Collection of milk samples and determination of SCC, from selected females which had been challenged with E. coli endotoxin 24 h earlier, at days 11 and 16.
- Collection of blood samples from selected females at days 7, 15 and 21.
- Bacteriological culture tests of all lactating females at day 14.
- Individual identification by toe-notching and sexing of non-fostered mice at day 12.
- Weaning mice at day 21.

To schedule these duties in each generation, several computer generated work lists were produced which detailed work to be performed each day.
MEASUREMENTS

SCC Determinations: It originally was planned to collect milk samples from lactating females of the three lines involved in the experiment, via a method of gland massage and pipette collection of milk. Because of the small quantities of the collected milk expected by this method, it was also decided to determine SCC in milk by standard direct microscopic procedures (127). Preliminary examination showed this method of milk collection to be tedious and time consuming. It became obvious that to carry out a selection experiment on milk SCC in three lines of mice with a reasonable number of observations, milk collection time had to be reduced substantially, and the quantity of milk obtained had to be increased to such a level that electronic somatic cell counting to be considered. A vacuum-operated milking device (Figure 1) similar to that described by McBurney et al. (89) was developed and used with success in preliminary experimentation. With vacuum at 100 mm Hg, the teat tube was placed firmly about the teat and vacuum applied by covering the air passage opening in the side of the device. For most mice, sample collection time was less than 1 min. Thus, milk collection time was reduced by approximately 20 fold using this device. In addition, a quantity of between 30 to 100 μl of milk, depending mainly upon the lactation day of the mouse, was easily obtained.

Since a reasonable quantity of milk was easily obtained by this method, it was decided to use electronic cell counting procedures for SCC determinations. The Virginia Dairy Herd Improvement milk processing laboratory, located in the Animal Science Building, Virginia Tech, operated three Fossomatic cell counting machines. This instrument is fully automatic. Heating to 40°C and subsequent shaking is the only preparation of the milk samples (88) required. The instrument mixes the individual sample and automatically takes .2 ml into a rack which is automatically led through the instrument. At the end of each test the resultant count, in number of cells/mm³ of milk, is printed on a strip of paper, and the result is also shown on a digital counter. The instru-

4 A/S N. Foss Electric, Hillerod, Denmark.
Figure 1. Diagrammatic representation of device used to obtain milk samples for somatic cell count determination.
ment has a capacity of 180 samples/h. It also produces very accurate counts (57, 68, 88) especially with fresh milk (100). However, because the smallest sample volume required by the instrument is 200 μl, it was decided to increase the volume of the milk samples obtained, by diluting them with physiological saline (9 g sodium chloride in 1000 ml distilled water).

Litters of lactating females to be sampled were removed from their dams for at least 3 h prior to sampling to increase sample yield. To expedite sample collection, females were anesthetized with methoxyflurane and injected subcutaneously with 1 USP oxytocin. Milk was then collected from individual teats using the milking device described previously. One to four teats in a priority order, R4, R5, L1, any other (as described by Chandler (29)) were sampled to obtain at least 30 μl of milk. Following sample collection, a microliter pipette was used to mix and transfer a standardized quantity of milk (25 μl) to a 1.5 ml microfuge tube containing 225 μl of physiological saline. Samples were mixed thoroughly by electrical mixer. Somatic cells were then immediately counted by Fossomatic cell counter. A control sample (containing only buffer) was used to adjust for machine inconsistency. Any negative number resulting from correction for buffer reading, as well as zero SCC were converted to 1 to permit logarithmic transformation. The SCC read by the instrument were then multiplied by 10 to adjust for the dilution effect, as well as by the factor 1000 to express the scores in number of cells/ml of milk. These standardized collection, dilution, mixing and counting procedures yielded high repeatability for split samples of the same mouse-day (r = .99, n = 49).

Total SCC for the lactation was the average SCC for individual days (9, 14 and 18), after transformation to log base 2 (155) and adjustment for stage of lactation (adjusted to day-14 basis). This adjustment was done because a large stage of lactation effect became apparent from generation zero. SCC at day-14 was slightly higher but those at day-18 much higher than SCC at day-9. The increase in SCC with day of lactation was studied up to generation 3, and shown to be consistent in all lines and generations and was significant (P < .01) by analysis of variance after accounting for linear and quadratic effects of milk yield (Appendix A). The effect of stage of lactation on SCC in mice is in contrast to the smaller increases in SCC with increasing stage of lactation reported in
dairy cattle (76). An original intention to use only two days’ counts was rejected after a low repeatability (.14) of log₂ SCC from the same female was found (Appendix B). In fact, this repeatability is similar to the .13 reported within herd and lactation for individual test days of first lactation cows (158).

**Milk Yield Determinations.** Individual day milk yield (days 8, 13, 17 and 20) was determined by the method described by Jara-Almonte and White (75). Litters were separated from their dams for a period of 6 h. During this time pups did not have access to food and water. Following separation, litters were weighed and their weight was recorded as ‘weight before suckling’. Then litters were returned to their dams and allowed to suckle for 1.5 h. Again litters did not have access to food or water. Litters were again weighed and their weight was recorded as ‘weight after suckling’. Milk yield for the day was determined as the difference between ‘after’ and ‘before’ weights of the litter. No adjustment for losses in urine and feces during the suckling period was made because after 6 h of starvation the alimentary tract of the pups could be expected to be almost empty (12). Differences between litter weights ‘after’ and ‘before’ of less than zero were set to zero. Observations with zero grams of milk yield at days 8 or 13 were discarded for the obvious reason that young can not survive without milk at this early stage. Observations with daily yield, at days 8, 13, 17 and 20, of more than the mean yield for that day + 3x the standard deviation for the day, were also discarded before any analysis involving milk yield was performed.

Total (21 days) milk production for each lactating female was estimated from yields at days 8, 13, 17 and 20 or days 8, 13 and 17 only (depending on the available information) using the appropriate regression equation described by Jara-Almonte (74). The equation making use of these 4 days’ milk yields, produces an accuracy of the estimation of total milk yield of .76. The equation making use of three days’ milk yields (8, 13 and 17) produces an accuracy of .67. Females producing no milk at days 8, 13 or 17 were excluded from analysis.

Materials and Methods
E. Coli Endotoxin Trials. To study line differences in ability to mobilize phagocytic cells, E. coli endotoxin (lipopolysaccharide) challenge trials were conducted, beginning in generation 3. In this generation, 18 females were chosen randomly from each line (6 per mating group) for testing on day 11 or 16 of lactation (9 each day). From generation 4, the number was increased to 24 per line (8 per mating group; 12 each day). Females were anesthetized and glands R4, R5, and L1 were washed with 70% alcohol and each injected with .05 ml endotoxin in physiological saline (.1 mg/ml). After 24 h, milk samples were collected and evaluated for SCC as described previously. Determinations of SCC of the same mouse from the previous non-challenge day (day 9 for challenge day 11; day 14 for challenge day 16) were used as controls to evaluate line differences in mobilization of SCC in response to endotoxin challenge. When the difference, challenge-control SCC, was negative or zero, this difference was converted to 1 so that logarithmic transformation was possible.

It should also be mentioned that to establish the appropriate concentration of endotoxin as well as the appropriate postinjection time of SCC determination, preliminary experimentation was undertaken. The concentrations examined in several mice were .02, .05, .1, .15 and .20 mg/ml. Concentrations less than .1 mg/ml did not cause substantial increase in milk SCC, while the higher concentrations (.15 and .20 mg/ml) caused extreme swelling of the gland and inability to collect milk samples in most mice. Thus, the .1 mg/ml concentration was established as the best concentration for the purposes of this experiment (seldom hardening of the gland and a reasonable and consistent increase in SCC). The postinjection time of milk SCC determination was also examined in 12 mice (Appendix C). SCC in milk were determined before intramammary injection with endotoxin and then at various postinjection periods using milk taken from the same glands. There was an average 55-fold increase in SCC (P < .01) 24 h after injection and a sequential decrease in SCC and up to 72 h after injection, at which time SCC numbers returned to their original (preinjection) levels (P > .90). Thus, the 24 h postinjection time of SCC determination was established as the time of highest increase in SCC. This small experiment also showed that carryover effects
of endotoxin injected at lactation day 15 (for the challenged mice to be sampled at day 16) on SCC of milk samples collected regularly on day 18 are probably very small.

**Blood Samples.** Blood samples were obtained from lactating females beginning in generation 2. In generations 2 and 3, 18 females were assigned randomly from each line for blood sampling on day 7, 15, or 21 (6 each day). From generation 4, the number sampled was increased to 24 (8 each day). Females chosen for blood sampling were the same mice assigned for endotoxin challenge trials (starting in generation 3). After anesthetizing the female, .02 ml of blood was obtained via tail vein lance and transferred by capillary tube to 1.98 ml glacial acetic acid solution anticoagulant. Total cell count was determined by haemocytometer. In addition, slides were prepared for differential microscopic counting of PMN, lymphocytes, monocytes, and eosinophils. Blood samples (for total and differential counting) were processed immediately after collection by the Pathology Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine.

**Bacteriological Cultures.** To study line differences in naturally occurring rates of infection, routine bacteriological culture tests of all lactating females at day 14 of lactation were performed at generations 5 and 6. Mice were anesthetized and glands R4, R5 and L1 were washed with 70% alcohol and were gently massaged. Parts (collection vial, rubber stopper and capillary tube) of the milking device (Figure 1) contacting teats and milk of females to be tested, were sterilized. Milk samples collected, were mixed thoroughly and 10 μl of milk was removed by a microliter pipette and placed on a plate containing blood agar. In general, plating and culturing procedures were according to "Microbiological Procedures for the Diagnosis of Bovine Mastitis" published by the National Mastitis Council (24). Plates with bacterial growth were examined and identified by the Microbiology Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine.
Selection for Low and High Milk SCC

In lines selected for decreased (LSCC) and increased (HSCC) SCC in milk, entire litters were selected based on the average breeding value of their parents.

The basic measure of performance in breeding values was the average $\log_2$ of one to three (as available) milk SCC measurements during the lactation, adjusted for stage of lactation ($\log_2$ SCC of days 9 and 18 adjusted to day-14 basis), and deviated from the appropriate line, generation mean:

$$X_{ijk} = \overline{Y}_{ijk} - \overline{Y}_{ij}$$

where:

- $X_{ijk}$ = the basic measure of performance of the $k$th female in the $j$th line and $i$th generation.
- $\overline{Y}_{ijk}$ = the mean $\log_2$ SCC determination for the $k$th female in the $j$th line and $i$th generation.
- $\overline{Y}_{ij}$ = the mean $\overline{Y}_{ijk}$'s for all females in the $j$th line and $i$th generation.

For sires of litters, the breeding value was computed from a 6-member selection index (67) of the form:

$$I = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6$$

where:

- $X_1$ = the basic measure of performance of the sire's dam.
- $X_2$ = the basic measure of performance of the sire's full sisters (mean).
- $X_3$ = the basic measure of performance of the sire's maternal granddam.
- $X_4$ = the basic measure of performance of the sire's paternal granddam.
- $X_5$ = the basic measure of performance of the full sisters (mean) of the sire's sire.
- $X_6$ = the basic measure of performance of the full sisters (mean) of the sire's dam.
- $b$'s = selection index weights based on amount and source of information.
Identical relatives of the dam, together with her own SCC determination, were used to compute breeding values for dams of litters (i.e., a 7-member selection index). Since three generations were required to obtain measurements for a complete complement of relatives, parents of the three initial generations were selected on partial indexes from measurements of available relatives. In line LSCC, litters from parents having lowest average breeding value were chosen as parents, while in HSCC, litters having highest average breeding value for SCC were chosen. To minimize effects of inbreeding, a maximum of four males and four females were used as parents from any single litter. This restriction and an average standardized litter size of approximately 10 mice, yielded a selection intensity of approximately 25%. Parents in the control line were selected at random and matings in all lines were at random except for the avoidance of full-sib matings. With a full complement of relatives and assuming \( h^2 = 0.10 \) for \( \log_2 \) SCC determinations, theoretical accuracies for these indexes are approximately 0.4 and 0.5 for males and females. In addition based on the selection intensity applied (25%), a theoretical change of approximately 0.17 standard deviations in average \( \log_2 \) SCC per generation was expected.

Finally, after selection of parents for the next generation was completed, all remaining mice were discarded.

Statistical Procedures

**PHENOTYPIC RELATIONSHIPS OF MILK SCC WITH MILK YIELD, TOTAL LEUKOCYTES IN BLOOD, PERCENTAGE OF PHAGOCYTIC CELLS, RESPONSE TO E. COLI ENDOTOXIN CHALLENGE, FITNESS AND TYPES OF BACTERIA NATURALLY OCCURRING IN MILK**

Phenotypic correlations between milk SCC and milk yield, total leukocyte count in blood, percentage of phagocytic cells in blood, response to E. coli endotoxin challenge, number of young born
and percentage of young surviving to weaning were computed by multivariate analysis of variance. The model included as dependent variables the primary character (milk SCC), as well as all the secondary traits mentioned above. The analysis was performed within generation, line and mating group. Correlation coefficients between milk SCC and secondary traits as well as among secondary traits were computed and tested for significance by t-tests.

**Milk Yield:** The relationship between SCC and milk yield was determined in two ways:

1) by regressing lactation milk yield on lactation average milk SCC. In this analysis the following model was used:

\[
Y_{ijkl} = a + G_i + L_j + M_{k(\ell)} + (GL)_{ij} + b_1 S_{ijkl} + b_2 S_{ijkl}^2 + b_3 S_{ijkl}^3 + e_{ijkl}
\] (1)

where:

- \(Y_{ijkl}\) = the lactation milk yield of the \(i\)th female from the \(i\)th generation in the \(j\)th line and \(k\)th mating group.
- \(a\) = intercept.
- \(G_i\) = the effect of the \(i\)th generation (\(i = 0\ldots7\)).
- \(L_j\) = the effect of the \(j\)th line (\(j = 1\ldots3\)).
- \(M_{k(\ell)}\) = the effect of the \(k\)th mating group within the \(i\)th generation (\(k = 1\ldots3\)).
- \((GL)_{ij}\) = the effect of the interaction of the \(i\)th generation with the \(j\)th line.
- \(S_{ijkl}\) = the lactation (log2, day adjusted) SCC of the \(i\)th dam from the \(i\)th generation in the \(j\)th line and \(k\)th mating group.
- \(S_{ijkl}^2\) = the quadratic SCC of the \(i\)th dam from the \(i\)th generation in the \(j\)th line and \(k\)th mating group.
- \(S_{ijkl}^3\) = the cubic SCC of the \(i\)th dam from the \(i\)th generation in the \(j\)th line and \(k\)th mating group.
- \(b\)'s = regression coefficients.
- \(e_{ijkl}\) = random error.

In this model lactation milk yield for each female was computed as indicated previously, using a regression equation (74) and daily milk yield (days 8, 13, 17 and 20) to predict lactation yield.
Lactation SCC for each female was the average log2 of three SCC (days 9, 14 and 18) after adjustment for stage of lactation. Generation number, line and mating group of the dam were treated as classification variables. The purpose of this first analysis was to determine the relationship between lactation milk yield and lactation SCC. Regressions of lactation yield on linear, quadratic and cubic lactation SCC were fit sequentially after other effects in the model and the effects tested for significance by F-tests. Regression coefficients were tested by t-tests.

2) by regressing daily milk yield (days 8, 13, and 17) on nearest lactation day log2 SCC (days 9, 14, and 18) within line, generation, and female. In this analysis the following model was used:

\[ Y_{ijklm} = a + G_i + L_j + M_{k(i)} + (GL)_{ij} + D_{li(k)} + W_m + b_1 S_{ijklm} + b_2 S^2_{ijklm} + b_3 S^3_{ijklm} + e_{ijklm} \]  

(2)

where:

- \( Y_{ijklm} \) = the milk yield of the \( f_{th} \) female from the \( l_{th} \) generation in the \( j_{th} \) line and \( k_{th} \) mating group on lactation day 8 or 13 or 17, and with SCC on the nearest \( m_{th} \) lactation day.
- \( a \) = intercept.
- \( G_i \) = the effect of the \( l_{th} \) generation (\( i = 0...7 \)).
- \( L_j \) = the effect of the \( j_{th} \) line (\( j = 1...3 \)).
- \( M_{k(i)} \) = the effect of the \( k_{th} \) mating group within the \( l_{th} \) generation (\( k = 1...3 \)).
- \( (GL)_{ij} \) = the effect of the interaction between the \( l_{th} \) generation with the \( j_{th} \) line.
- \( D_{li(k)} \) = the effect of the \( l_{th} \) dam from the \( l_{th} \) generation in the \( j_{th} \) line and \( k_{th} \) mating group.
- \( W_m \) = the effect of the \( m_{th} \) day of lactation (nearest to day 8 or 13 or 17) on which the SCC for the \( l_{th} \) female was determined (day 9 or 14 or 18) (\( m = 1...3 \)).
- \( S_{ijklm} \) = the SCC (log2, day adjusted) of the \( l_{th} \) dam from the \( l_{th} \) generation in the \( j_{th} \) line and \( k_{th} \) mating group on the \( m_{th} \) day of lactation.
- \( S^2_{ijklm} \) = the quadratic SCC of the \( l_{th} \) dam from the \( l_{th} \) generation in the \( j_{th} \) line and \( k_{th} \) mating group on the \( m_{th} \) day of lactation.
- \( S^3_{ijklm} \) = the cubic SCC of the \( l_{th} \) dam from the \( l_{th} \) generation in the \( j_{th} \) line and \( k_{th} \) mating group on the \( m_{th} \) day of lactation.
- \( b's \) = regression coefficients.
- \( e_{ijklm} \) = random error.
The effects of generation, line, mating group, day of SCC determination and dam were treated as classification variables and the effect of dam was absorbed. Regression coefficients $b_1$, $b_2$ and $b_3$ determined from this model, were used to produce the relationship curve between milk yield and $\log_2$ SCC:

$$Y_{ijklm} = a + \hat{b}_1 S_{ijklm} + \hat{b}_2 S_{ijklm}^2 + \hat{b}_3 S_{ijklm}^3$$

where b's are estimates from the previous model.

**Total Leukocytes in Blood, Percentage of Phagocytic Cells and Response to Endotoxin Challenge:**

Relationships between milk SCC, total leukocyte counts in blood and percentage of phagocytic cells were studied using the model (2) on page 57. Dependent variables were the total leukocyte count in blood ($\log_2$) or percentage of phagocytic cells (PMN and monocytes as a percentage of total leukocytes in blood). Independent variables were those in model (2) except that dam was not included in the model (only one record per dam was available) and $S_{ijklm}$ was $\log_2$ milk SCC (adjusted for stage of lactation) of the $i^{th}$ female sampled on the day (9 or 14 or 18) nearest to the day on which total leukocyte counts and percentage of phagocytic cells were determined (day 7 or 15 or 21).

The relationship between milk SCC and response to E. coli endotoxin challenge ($\log_2$ of the difference between milk SCC of challenged - control glands) was also studied by the model (2) on page 57. The dependent variable was the response to endotoxin of the $i^{th}$ female on a certain lactation day (11 or 16) and the independent variable $S_{ijklm}$ represented the $\log_2$ milk SCC (adjusted for stage of lactation) of the $i^{th}$ female sampled on a day (9 or 14) nearest to the day on which the female was challenged. Again, the effect of dam was not included in the model (one record per dam available).
Fitness: Effects of milk SCC on two elements of fitness were examined. These elements included reproductive performance and survival to weaning. Reproductive performance was characterized by percentage of successful matings and number born per litter. Percentage of successful matings was defined as the number of litters produced in a line divided by the number of scheduled matings (approximately 50 in selected lines and 36 in the control line) in the line. Survival to weaning was defined as the percentage of young in a litter (including any fostered mice) that survived to weaning (day 21 of lactation). Model (1) on page 56 was used to examine relationships of milk SCC with number of young born and survival to weaning. Also, line-generation-mating group means for percent successful matings were used as dependent variables in model (1) page 56 (the model did not include SCC) and line differences in percent successful matings were examined by F-test.

Types of Bacteria Naturally Occurring in Milk: The relationship between milk SCC and types of bacteria naturally occurring in milk of mice in the three lines involved in the experiment was studied in two ways:

1) Mice were grouped according to infection status (infected or not) with respect to one or more of the following types of bacteria:

- Bacillus sp.
- Pasteurella sp.
- Corynebacterium pseudodiphtheriticum.
- Micrococcus sp.
- Streptococcus fecalis.
- Pasteurella pneumotropica.
- Staphylococcus saprophyticus.
- Corynebacterium sp.
- Staphylococcus aureus.
- Fecal streptococcus sp.
• Staphylococcus intermedius.
• Neisserio sp.
• Flavobacterium.
• Staphylococcus epidermis.

An ANOVA was then performed and differences between the two classes of mice in lactation day 14 \( \log_2 \) milk SCC (bacterial cultures were performed on day 14) and in \( \log_2 \) of lactation milk SCC were tested by F-tests. The model used included effects of generation, line, mating group, and the interaction generation x line.

2) Mice were grouped in the following five classes: a) not infected b) infected with Bacillus species c) infected with Corynebacterium species (C. pseudodiphtheriticum etc.) d) infected with Pasteurella species (P. pneumotropica etc.) and e) infected with any one or more of the remaining species identified. The same ANOVA model which was previously described, was then used to test for differences among the means of \( \log_2 \) milk SCC (day 14 SCC and lactation SCC) of the five groups of mice. Orthogonal contrasts among the various groups were computed and tested.

RESPONSE TO SELECTION

Direct Response to Selection for Low and High Milk SCC

Line and generation means for average \( \log_2 \) SCC determinations (1 to 3 measurements per female) were computed for the lines LSCC, HSCC and CSCC. Means of the selected lines were deviated from control line means and regressed on generation number (8 generations). The regression coefficients computed, represented the average response to selection for low and high milk SCC per generation (54). Also, line-generation means for \( \log_2 \) SCC of line LSCC were deviated from means of line HSCC and the deviations were regressed on generation. All regression coefficients were
tested for significance by t-tests. The symmetry of response to selection in the two selected lines was examined by comparing the slopes of two fitted linear regression lines.

In addition, the regression of performance on genetic selection differential was computed. This regression is similar to a realized heritability except that heritability here was already accounted for (in the determination of the selection indexes), and thus the expected value of this regression is actually 1. Line and generation means for log₂ milk SCC of line LSCC were deviated from the corresponding generation means of line HSCC. Also, the genetic selection differential for each selected line was computed by deviating the line-generation mean breeding value of selected parents from the appropriate line-generation mean breeding value of all individuals in that line-generation. Selection differentials of line LSCC were then deviated from those of line HSCC, and the cumulative selection differential over seven generations was computed. The regression of deviated line-generation means of SCC on the cumulative selection differential was then computed.

The approximate rate of inbreeding was computed for each line per generation by the procedure outlined by Wright (181) as follows:

\[ F = \frac{1}{8N_f} + \frac{1}{8N_m} \]

where \( N_m \) and \( N_f \) are the numbers of males and females per generation, respectively.

**Correlated Responses in Milk Yield, Total Leukocytes in Blood, Percentage of Phagocytic Cells, Response to E. Coli Endotoxin Challenge and Fitness**

To determine correlated responses for the seven secondary traits examined (milk yield, total leukocyte count in blood, percentage of phagocytic cells, response to E. coli endotoxin challenge, percentage littering, number of young born and percentage of young surviving to weaning), line-generation means were computed and means of the selected lines were deviated from those of the
control line. The deviated means were then regressed 1) on generation number and 2) on the deviated means of the primary character (milk SCC) (genetic regressions). Deviations of the form HSCC - LSCC for the secondary traits were also computed and regressed on generation number. Slopes were tested for significance by t-tests.
Results and Discussion

Distribution of SCC in Milk of Laboratory Mice

To our knowledge no study has been published on SCC in milk of laboratory mice. It was therefore considered useful to compute the distribution of SCC in milk of mice and compare it with the distribution of SCC in milk of cows. Table 6 presents distributions of SCC in mice from all data (days 9, 14 and 18) as well as from data obtained at lactation days 9 and 14 only. In addition, Table 6 presents typical distributions of SCC from Holstein cows (76, 152). Distribution of SCC from all sampling days indicated that counts in mice were approximately 5x those in cows, except for an excess of extremely high counts in mice. An excess of extremely high counts in mice on sampling day 18 is demonstrated by the distribution of SCC from days 9 and 14 only. For days 9 and 14 the distribution of SCC in mice was very nearly 4x those in Holstein cows.
Table 6. Distribution of somatic cell counts (SCC) in milk of cows (76,152) and mice.

<table>
<thead>
<tr>
<th>Cows</th>
<th>All data(^1)</th>
<th>Days 9, 14(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC(^3)</td>
<td>(%)</td>
<td>SCC(^3)</td>
</tr>
<tr>
<td>≤ 100</td>
<td>36</td>
<td>≤ 500</td>
</tr>
<tr>
<td>100-200</td>
<td>21</td>
<td>500-1000</td>
</tr>
<tr>
<td>200-300</td>
<td>12</td>
<td>1000-1500</td>
</tr>
<tr>
<td>300-400</td>
<td>8</td>
<td>1500-2000</td>
</tr>
<tr>
<td>400-500</td>
<td>5</td>
<td>2000-2500</td>
</tr>
<tr>
<td>&gt; 500</td>
<td>18</td>
<td>&gt; 2500</td>
</tr>
</tbody>
</table>

1. n = 2727.
2. n = 1818.
3. Cells/mm\(^3\)

Results and Discussion
Lactation Curve of Mice

A lactation curve (Figure 2) was developed by regressing individual day milk yields (days 8, 13, 17 and 20) on day of lactation, within generation, line and mating group. The model included as independent variables linear and quadratic effects of day. Both linear and quadratic effects were highly significant ($P < .001$), with the model accounting for 28% of the observed variation in milk yield ($n = 3603$). The general pattern of the lactation curve in mice, as derived in this study, is similar to the pattern of the lactation curve in Holstein cows with peak yield occurring early in lactation. According to the present curve, peak yield (2.06 gms) occurred at about day 7. However, since the first yield determination was at day 8, the peak day falls in the projected (by the model) region of the curve. Enzeman (51) reported a similar quadratic shape of the lactation curve in mice, but the peak occurred at about day 10 postpartum. Hanrahan and Eisen (66) also determined the lactation curve in mice. They reported a peak yield (1.8 gms/day) between day 12 and 13. However, these authors determined daily milk yield by direct mechanical milking procedures. Finally, Jara-Almonte and White (75) described lactation curves determined by second and third degree polynomials which accounted for 92.3 and 98.6% of observed variation in milk yield. Peaks of the two lactation curves occurred at days 13 and 14 for the second and third degree polynomial. However, the maximal yield per dam per day (3.1 gms) in this study (75), was higher than maximal yield reported in the present study (2.06 gms) as well as higher than maximal yield (1.8 gms) reported in (51).
Figure 2. Lactation curve of mice.
Phenotypic Relationships of Milk SCC with Milk Yield, Total Leukocytes in Blood, Percentage of Phagocytic Cells, Response to E. Coli Endotoxin Challenge, Fitness and Types of Bacteria Naturally Occuring in Milk

Phenotypic correlations between milk SCC and correlated traits, as well as among correlated traits, were computed within line, generation and mating group and results are in Table 7.

**Milk Yield**

Lactation Milk Yield.

A small negative correlation (-0.01) between lactation milk yield and log2, lactation milk SCC was found. This correlation (although non-significant (P > .84)) agrees in general with results reported in studies with dairy cattle (34, 76). In addition, Shook (156) recently summarized phenotypic correlations, between yield and SCC, which ranged from -.10 to -.20. The same author concluded that the negative correlation is mediated by mastitis which elevates SCC and reduces yield through damage to secretory epithelium (156). The closer to zero correlation reported in the present study might be due to biological differences between cows and mice, to the fact that mice in our study were not found to suffer from major mastitis infections and/or sampling.

In addition, the relationship between lactation milk yield and lactation SCC, was studied by regressing (model 1 page 56) yield on linear, quadratic and cubic SCC (fit sequentially after other effects were in the model). Results are in Table 8. In addition, Figure 3 shows the phenotypic relationship between yield and SCC as this relationship was determined by model 1 (Table 8). Results in Table 8, indicate a negative linear association between milk yield and SCC determined by model 3. These results are consistent with the negative relationship reported between yield and SCC (within cow) in dairy cattle (34) and indicate animals with higher SCC in milk produce less.
Table 7. Phenotypic correlations between milk SCC and correlated traits (n = 233).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Milk SCC</th>
<th>Milk Yield</th>
<th>Blood SCC</th>
<th>% Phag. Cells</th>
<th>Resp. to End. Chall.</th>
<th>Number Born</th>
<th>Survival¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Yield²</td>
<td>-0.01</td>
<td>-0.09</td>
<td>-0.06</td>
<td>-0.14*</td>
<td>0.08</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Blood SCC⁴</td>
<td></td>
<td></td>
<td></td>
<td>0.27**</td>
<td>0.03</td>
<td>0.03</td>
<td>-0.13</td>
</tr>
<tr>
<td>% Phag. Cells⁵</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.11</td>
<td>0.12</td>
<td>-0.18**</td>
</tr>
<tr>
<td>Resp. to End. Chall.⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td></td>
<td>-0.07</td>
</tr>
<tr>
<td>Number Born⁷</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.05</td>
</tr>
</tbody>
</table>

* Correlation coefficient significant at level .05.
** Correlation coefficient significant at level .01.
1. Percentage of young surviving to weaning.
2. Log₂ of lactation average milk SCC.
3. Lactation milk yield.
4. Log₂ of total leukocytes in blood.
5. Percentage of phagocytic cells (PMN and monocytes) in blood.
6. Response to E. coli endotoxin challenge (page 52).
7. Number of young born in a litter.
### Table 8. Regression of lactation milk yield on lactation milk SCC. Coefficients of regression, SEs, and $R^2$ values ($n = 792$).

<table>
<thead>
<tr>
<th>Models</th>
<th>Linear$^1$</th>
<th>Quadratic$^1$</th>
<th>Cubic$^1$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1$^2$</td>
<td>0.430</td>
<td>0.064</td>
<td>-0.003</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>$\pm 15.308$</td>
<td>$\pm 0.805$</td>
<td>$\pm 0.014$</td>
<td></td>
</tr>
<tr>
<td>Model 2$^3$</td>
<td>3.504</td>
<td>-0.099</td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>$\pm 1.880$</td>
<td>$\pm 0.049$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3$^4$</td>
<td>-0.276</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>$\pm 0.136$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Linear, quadratic and cubic log$_2$ lactation milk SCC fit sequentially after other effects.

2. No regression was significant ($P > .83$).

3. Regression of linear SCC was non-significant ($P > .05$).
   Regression of quadratic SCC was significant ($P < .05$).

4. Regression of linear SCC was significant ($P < .05$).
Figure 3. Phenotypic relationship between lactation milk yield (gms) and lactation average milk SCC (log₂).
milk. However, the pattern in Figure 3, with increasing milk yield as SCC increase up to about 260,000 cells/ml (18 in log) and then decreasing yield with increasing SCC, is in contrast to results (within cow) in cattle (76) where there is a continuous decrease of yield with increasing SCC (although there is a higher marginal decrease in milk yield at low levels of SCC (76)). The exact reason for this tendency in mice is not known. However, one explanation might be that levels of cells which could be characterized as detrimental for milk yield in cows, might not affect milk yield in mice. In a recent study (55) dealing with a data set in which more than half the quarter samples had SCC of less than 200,000 cells/ml, the authors found that with every doubling of cell count there was a loss of .12 kg milk in comparisons within cow between udder halves. The authors concluded that perhaps parenchymal damage is associated with milk cell concentrations (< 500,000 cells/ml) considered to be well within the range of normal milk. In another study (128), loss of milk yield at counts of less than 100,000 cells/ml was reported. Similar results and conclusions have been reported by other researchers (4, 156, 167). However, the distribution of SCC in milk of mice in Table 6 shows that the concentration of SCC in mice is 4 to 5x greater than that in dairy cows. This indicates that a level of SCC in milk of mice of 260,000 cells/ml or lower might be considered as too low to cause any damage in the secretory epithelium of the mammary gland of mice. However, when the level of SCC increased substantially (Figure 3), a detrimental effect in lactation milk yield was observed. Of course this explanation is not complete. For instance, if we accept the idea that low levels of SCC (lower than 200,000) are too low to cause any damage to the secretory cells of mice, we still cannot explain why there should be an increase in yield with increasing SCC at these low levels of SCC. The only reason we can give at the moment is sampling.

Small positive correlations (about .20) were found between milk yield and two fitness traits (number born and % survival). However, these correlations were highly significant (P < .005). Presumably, dams producing larger litters also had higher lactation milk production and tended to wean more young than dams producing smaller litters (dispite the fact that litters were standardized).
Daily Milk Yield.

The phenotypic relationship between milk yield and milk SCC was also studied by regression (model 2 page 57) of daily yield (days 8, 13 and 17) on daily SCC (days 9, 14 and 18) (n = 2084; R² = 0.50). The analysis was within generation, line, mating group, day of sampling and dam. The effect of dam was absorbed. The regression coefficients computed for linear, quadratic and cubic SCC were 1.185±0.887, -0.064±0.049 and 0.001±0.001 respectively. In this analysis the only significant regression was that of linear SCC (P < .05). Figure 4 shows the relationship between daily milk yield and daily SCC. The negative association between individual day milk yield and SCC over much of the range in SCC is obvious and similar to the relationship between lactation yield and SCC. However, there are two points that need to be emphasized here. The first is that the critical level of SCC above which individual day milk yield is decreased was much lower (about 57,000 cells/ml) as compared to Figure 3 for total lactation yield (260,000 cells/ml). The second is the tendency of yield to increase (Figure 4) when daily milk SCC increase beyond 8.5 million cells/ml. This phenomenon, as well as the increase of yield with increased SCC at very low levels of SCC (lower than 50,000 cells/ml), may be attributed to sampling.

TOTAL LEUKOCYTES IN BLOOD

A small negative correlation (-0.09) between milk SCC and blood SCC was observed. This correlation was non-significant (P > .20) (Table 7). The phenotypic relationship between milk SCC and blood SCC was also studied by regressing (model 2 page 57) leukocytes in blood on leukocytes in milk within generation, line, mating group and day of sampling (n = 239; R² = 0.26). Regression coefficients for linear, quadratic and cubic milk SCC (computed sequentially after other effects were fit in the model) were -0.001±0.018, 0.007±0.005, and zero respectively. No regression was significantly different than zero (P > .15). To our knowledge there have been no studies published on the relationship between SCC in milk and SCC in blood. However, the finding in the present study of no relationship between the two variables is a little surprising. One could expect that uninfected mice (it will be shown later that no major mastitis pathogens were found in these mice) with high
Figure 4. Phenotypic relationship between daily (days 8, 13, and 17) milk yield (gms) and nearest lactation day log₂ milk SCC (days 9, 14, and 18).
numbers of milk SCC have also high numbers of cells in their blood and vise-versa. This expectation would of course be based on the assumption that migration of leukocytes from blood to milk (under non-infection conditions) is proportional to the number of leukocytes available in the bloodstream. However, the extent to which this assumption is true is not known. The mechanism of leukocytic migration under normal (non-infected) conditions might very well constitute a separate genetic trait. Then two animals with similar blood leukocytic pools might have (for unknown reasons) very different milk leukocytic pools.

In addition, a small, but highly significant (P < .01) positive correlation (0.27) was found between total cell counts in blood and percentage of phagocytic cells in blood. This result indicates that female mice with higher total cell count in blood tended on the average to have also higher percentages of phagocytic cells (PMN and monocytes) in blood, in spite of the fact that the former is the denominator in determining percentage of phagocytic cells. This may indicate that higher blood SCC were associated with increased levels of infection (not necessarily mammary) of dams.

**PERCENTAGE OF PHAGOCYTIC CELLS**

It has been mentioned that PMN and macrophages are the most important leukocytes in phagocytosis (115, 124) (although other cells, i.e., lymphocytes, also play an important role in udder defense). In the present study, percentages (% of total leukocytes in blood) of PMN and monocytes (the circulatory form of a macrophage) were combined and the two types of cells were called phagocytic cells. A small negative correlation (-0.06) between this trait and milk SCC (Table 7) was observed. This correlation was non-significant (P > .36). The phenotypic relationship between milk SCC and phagocytic cells was also studied by regressing (model 2 page 57) percentage of phagocytic cells on milk SCC within line, generation, mating group and day of sampling. Regressions (n = 239; R² = 0.20) for linear, quadratic and cubic SCC (fit sequentially after all other effects in the model) were -0.058 ± 0.401, 0.047 ± 0.117 and 0.002 ± 0.039 respectively. No regression was significantly different than zero (P > .68).
The highly significant \((P < .01)\) negative correlation \((-0.18)\) between percentage phagocytic cells in blood and percent survival and the negative correlation between total blood cell count and percent survival \((-0.13)\) also may suggest greater levels of infection in dams with higher cell counts and increased percentage of phagocytic cells.

**RESPONSE TO CHALLENGE WITH ESCHERICIA COLI ENDOTOXIN**

Several studies \((140, 148, 167)\) have reported that E. coli endotoxin injected in the mammary gland of cows, has caused massive migrations of leukocytes from the blood stream, through the secretory tissue into the alveolus of the gland. In addition, preliminary experimentation in this study showed an average 55-fold increase in milk SCC of 12 mice 24h postinjection with \(100\mu g/ml\) E. coli endotoxin (Appendix C.). Furthermore, it is known \((101)\) that phagocytic competence on a systemic level depends not only on the ability of an individual leukocyte to phagocytose but also on the total number of cells available in the infected gland. An objective in this study was to determine whether natural variation in milk SCC (non-infected status) in mice, is associated with variation in ability to elevate SCC in milk, when the animal was injected with bacterial endotoxin.

A small, but significant \((P < .05)\) negative correlation \((-0.14)\), was found (Table 7) between response to E. coli endotoxin challenge and milk SCC, indicating that dams with inherently higher milk SCC responded less to endotoxin challenge than dams with lower milk SCC. This is a surprising result because one might logically expect the opposite to be true. However, response to live bacteria and response to bacterial products (i.e endotoxin) might be less than perfectly correlated measures of response.

Another analysis in this section was similar to analyses reported in the previous two sections. The difference in milk SCC between challenged and control glands (in the same animal) was regressed (model 2 page 57) on routinely determined (not challenged) milk SCC within line, generation, mating group and day of sampling. Regressions \((n = 226; R^2 = 0.28)\) for linear, quadratic and cubic SCC (fit sequentially after other effects in the model) were \(-0.384 \pm 0.147, -0.108 \pm 0.040\) and zero.
respectively. Regressions of linear and quadratic SCC were highly significant ($P < .01$). Figure 5 shows the phenotypic relationship between response to E. coli endotoxin challenge and milk SCC. The relationship curve indicates that at low levels of milk SCC ($< 65,000$ cells/ml) the ability of a mouse to elevate its milk SCC after endotoxin challenge, increases with increased milk SCC. However, at very high levels of milk SCC ($> 525,000$ cells/ml) the ability of response to challenge decreases with increased milk SCC. Overall, this figure indicates that low or moderate levels of milk SCC are preferred over high levels with regard to response to endotoxin.

A small positive correlation ($0.11$) between response to challenge and percentage phagocytic cells in blood also was observed (Table 7). This correlation was nearly significant ($P < .10$). This result indicates that dams with higher percentages of phagocytic cells in blood, also tended to have slightly greater response to endotoxin challenge than dams with lower percentages.

**FITNESS**

Phenotypic relationships between three components of fitness and milk SCC were studied. Fitness traits examined were percentage of dams littering, number of young born (alive) in a litter and percentage of young surviving to weaning (including fostered pups). These same traits have been studied as correlated responses in many selection experiments with mice (48, 85, 134, 182), to characterize fitness in selected and control lines and study the action of natural selection throughout the experiment. In addition, litter size (a trait similar to number born) has been studied as a direct selection trait in other studies (13, 19, 53).

A small positive ($0.08$) correlation between milk SCC and number born, and a small negative ($-0.04$) correlation between milk SCC and percentage surviving to weaning were found (Table 7). Both correlations were non-significant ($P > .20$).

The phenotypic relationship between milk SCC and number born alive ($n = 938; R^2 = 0.05$) as well as that between milk SCC and percentage of young surviving to weaning ($n = 909; R^2 = 0.07$), were
Figure 5. Phenotypic relationship between response to endotoxin challenge (log₂ SCC) and milk SCC (log₂).

Results and Discussion
also studied by regression analysis, within line, generation and mating group. Regression coefficients for linear, quadratic and cubic SCC (fit sequentially after other effects in the model) for number born were \(-0.014 \pm 0.054\), \(-0.016 \pm 0.020\) and \(-0.006 \pm 0.006\) respectively. Regression coefficients for linear, quadratic and cubic SCC for percentage survival were \(-0.001 \pm 0.001\), zero and zero respectively. No regression was significant \((P > .14)\) implying that milk SCC and these fitness traits are independent characters. No study has been published on the subject so a comparison of results is not possible.

In addition, to study line differences in percent littering, line-generation-mating group means for percent of successful matings were used as dependent variables in a model including as independent variables, line, generation, mating group and the interaction line x generation. The analysis (Table 9) showed no significant effect of the line on percent survival \((P > .37)\) suggesting no relationship between this trait of fitness and milk SCC (lines differed in SCC as will be shown).

**TYPES OF BACTERIA NATURALLY OCCURRING IN MILK**

Major mastitis causing microorganisms that have been isolated in cattle include the contagious pathogens Staphylococcus aureus and Streptococcus agalactiae (162, 163), as well as the environmental pathogens Staphylococcus epidermidis, Streptococci other than agalactiae, Coliforms (E. coli, Klebsiella sp. etc.) and Corynebacterium bovis (21, 46, 119). In addition, Staph. aureus, Strep. agalactiae, Coryn. pyogens, E. coli and Pseudomonas aeruginosa are some of the bacterial species that have been used (9, 10, 29) with success in investigating intramammary infections in mice.

In the present study, no live bacteria were used for intramammary infections (although inoculations are planned in the near future) of the mice involved. Bacterial cultures were performed for the purpose of studying the relationship between milk SCC and types of bacteria naturally occurring in milk of mice in the three lines involved in the experiment. Table 10 shows the types and frequencies of bacteria isolated from milk of 169 mice in two generations (5 and 6) of this experiment. Obviously, no major mastitis causing pathogens in cattle such as Strep. species (except Strep. fecalis
Table 9. Analysis of variance. Dependent variable: Percentage of successful matings ($n = 71, R^2 = 0.67$)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td>2</td>
<td>0.0132</td>
</tr>
<tr>
<td>Generation</td>
<td>7</td>
<td>0.0421</td>
</tr>
<tr>
<td>Mating Group (Gen.)</td>
<td>16</td>
<td>0.0248</td>
</tr>
<tr>
<td>Line x Generation</td>
<td>14</td>
<td>0.0065</td>
</tr>
</tbody>
</table>
Table 10. Types and frequency of bacteria naturally occurring in milk of mice. (n = 169).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.</td>
<td>24.9</td>
</tr>
<tr>
<td>Pasteurella sp.</td>
<td>3.6</td>
</tr>
<tr>
<td>Corynebacterium pseudodiphtheriticum</td>
<td>3.0</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>3.6</td>
</tr>
<tr>
<td>Streptococcus fecalis</td>
<td>1.8</td>
</tr>
<tr>
<td>Pasteurella pneumotropica</td>
<td>6.5</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>0.6</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>21.3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.2</td>
</tr>
<tr>
<td>Fecal streptococcus sp.</td>
<td>1.2</td>
</tr>
<tr>
<td>Staphylococcus intermedius</td>
<td>0.6</td>
</tr>
<tr>
<td>Neisserio sp.</td>
<td>0.6</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>1.8</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>1.8</td>
</tr>
</tbody>
</table>
3/169 or 1.8%) and/or coliforms were detected. Staph. aureus and Staph. epidermis were found in only 2 and 3 cases respectively, out of the 169 cases studied. The two highest frequencies found were 24.9% (42/169) for Bacillus sp. (not known as a mastitis causing pathogen in cattle) and 21.3% (36/169) for Corynebacterium sp.. These results suggest that the mice involved in this study were free from major mastitis infections. This conclusion was consistent with results obtained by analyses of variance for the effects of infection status at day-14 (infected vs non-infected) on day-14 milk SCC (n = 133; $R^2 = 0.15$) where the two groups had equal mean SCC ($P > .54$) as well as with the effects of infection status on lactation milk SCC (n = 133; $R^2 = 0.20$) where the two groups had equal mean SCC ($P > .33$). In another analysis, differences in day-14 and lactation milk SCC among five groups of mice, infected with various microorganisms (page 60), were examined by orthogonal contrasts. No differences among the five groups were found either in day-14 milk SCC ($n = 133; R^2 = 0.18; P > .10$), or in lactation milk SCC ($n = 133; R^2 = 0.20; P > .13$), indicating that the bacteria identified did not cause any serious mastitis infections and/or increase in milk SCC.
Direct Response to Selection for Low and High Milk SCC

Choice of a character to be selected may be determined solely by availability of materials and ease of measurements (tail length, amount of white spotting etc.), by promise of practical applications (efficiency of food utilization, milk production, resistance to disease etc.), or a combination of the two. Selection for milk SCC rather falls in the third category. As mentioned earlier, the main objective of this study was to determine the feasibility of increasing genetic resistance to mastitis in dairy cows by selecting for high or low milk SCC. The biological organism used in this experiment was chosen because of cost and time efficiency. However, the methods of obtaining data for milk SCC were similar to methods used routinely in dairy cows (page 48).

Generation means for average log₂ milk SCC determinations (1 to 3 measurements per female) of lines HSCC, CSCC and LSCC are in Table 11. Means of SCC are expressed as number of cells/mm³ as well as logarithms (base 2). In addition, Figure 6 shows the direct response to selection for high and low milk SCC during the 7 generations of full-sib family selection. Selection of parents started at generation zero. Females of that generation were measured for milk SCC and evaluations of dams of litters were based on performance of the individual mouse. Evaluations of sires of litters were based on performance of their full-sisters. Litters were selected as parents of the following generation based on the average performance of their parents. Following this generation, the selection index evaluations for both sires and dams of litters included more information (page 54).

In all but two generations, line HSCC had higher mean SCC than either LSCC or CSCC. At generation zero line CSCC had higher mean SCC than either HSCC or LSCC. Also, at generation 3, line HSCC produced a mean SCC which was lower than the mean of either CSCC or LSCC. Breaking of linkages (31) between genes responsible for SCC and genes controlling traits which in general tend to resist changes in gene frequencies (for high or low SCC), may explain the lack of

Results and Discussion
Table 11. Milk SCC of lines HSCC, CSCC and LSCC.

| Generation | HSCC | | | CSCC | | | LSCC | | |
|------------|------|---------|------|-------|------|-------|------|-------|
|            | No. of mice | Mean leukocyte number | Log<sub>2</sub> leukocyte number | Mean | SD | No. of mice | Mean leukocyte number | Log<sub>2</sub> leukocyte number | Mean | SD | No. of mice | Mean leukocyte number | Log<sub>2</sub> leukocyte number | Mean | SD |
| 0          | 35 | 894 | 19.77 | 0.98 | 32 | 1124 | 20.10 | 1.12 | 42 | 711 | 19.44 | 1.11 |
| 1          | 48 | 392 | 18.58 | 1.37 | 33 | 318 | 18.28 | 1.07 | 45 | 316 | 18.27 | 0.88 |
| 2          | 44 | 574 | 19.13 | 1.24 | 31 | 547 | 19.06 | 1.18 | 43 | 397 | 18.60 | 1.11 |
| 3          | 44 | 578 | 19.14 | 1.59 | 33 | 645 | 19.30 | 1.05 | 43 | 628 | 19.26 | 1.60 |
| 4          | 40 | 999 | 19.93 | 1.66 | 30 | 907 | 19.79 | 1.03 | 39 | 768 | 19.55 | 1.48 |
| 5          | 45 | 503 | 18.94 | 1.95 | 29 | 460 | 18.81 | 2.07 | 40 | 368 | 18.49 | 1.70 |
| 6          | 39 | 1155 | 20.14 | 2.09 | 28 | 1071 | 20.03 | 1.15 | 48 | 768 | 19.55 | 1.94 |
| 7          | 27 | 1116 | 20.09 | 0.90 | 31 | 965 | 19.88 | 1.09 | 36 | 578 | 19.14 | 0.95 |

*No. of cells/mm<sup>3</sup> (anti-log of mean log<sub>2</sub> leuk. no.).
Figure 6. (a) Direct response to selection for low and high milk SCC (log$_2$). (b) Fitted linear regression lines.
response to selection in the initial stage of the experiment (3 generations) (Figure 6a). However, from generation 4 there was a consistent response in the expected directions in both selected lines, which continued to generation 7. During the 8 generations (24 months) of the experiment, the original (generation zero) difference in SCC between lines HSCC and LSCC of about 183,000 cells/ml, increased to approximately 538,000 cells/ml (almost 3x) (Table 11). Thus, at generation 8 line HSCC had a mean twice the mean milk SCC of line LSCC (1,116,000 vs 578,000 cells/ml). However, the average response per generation, after adjusting for environmental effects, was very small in both lines (0.039 ± 0.030 in log for line HSCC and -0.032 ± 0.043 for line LSCC) (Figure 6b). The symmetry of response to selection in the two selected lines was examined by comparing the slopes of two fitted linear regression lines (Figure 6b). These slopes were not significantly different (P > .05) ignoring signs. Another estimate of the average direct response to selection per generation, was computed as a regression of the deviation HSCC-LSCC on generation number. This regression was also small (0.071 ± 0.041). In addition, Figure 7 reveals that environment in general (although controlled) tended to favor increased milk SCC.

The genetic selection differential for each line was computed by deviating the line-generation mean breeding value of selected males and females from the appropriate line-generation mean breeding value of all individuals in that line-generation. Selection differentials of line LSCC were then deviated from those of line HSCC and these deviations are in Table 12. The regression of deviated line-generation means of SCC (HSCC-LSCC) on the cumulative selection differential produced a regression coefficient of 0.287 ± 0.214. Since the selection differentials used were breeding values with an assumed heritability of .10, the expected regression was 1. The divergence between the expected and computed regressions could be explained only on the basis that the assumed heritability (.10) was much higher than the true value.

Approximate rates of inbreeding per generation were computed for each line by the procedure outlined by Wright (181). These rates were 0.005 for each of the selected lines and 0.007 for the control line. These negligible inbreeding rates may have been observed as a result of the systematic avoidance of sib-matings in the selection program.
Figure 7. (a) Change over time in log₂ milk SCC of line CSCC. (b) Fitted linear regression line.

Results and Discussion
Table 12. Genetic selection differentials* for Log₂ milk SCC

<table>
<thead>
<tr>
<th>Generation</th>
<th>Genetic Selection Differential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.186</td>
</tr>
<tr>
<td>2</td>
<td>0.823</td>
</tr>
<tr>
<td>3</td>
<td>0.172</td>
</tr>
<tr>
<td>4</td>
<td>0.197</td>
</tr>
<tr>
<td>5</td>
<td>0.173</td>
</tr>
<tr>
<td>6</td>
<td>0.186</td>
</tr>
<tr>
<td>7</td>
<td>0.203</td>
</tr>
</tbody>
</table>

* Mean breeding value of selected (as parents) individuals, deviated from the mean breeding value of all individuals, in that line-generation. Deviations of the form HSCC-LSCC are presented here.
Correlated Responses in Milk Yield, Total Leukocytes in Blood, Percentage of Phagocytic Cells, Response to E. Coli Endotoxin Challenge and Fitness

**MILK YIELD**

Generation means and standard deviations for lactation milk production (in gms) of lines HSCC, CSCC and LSCC are in Table 13. In addition, Figure 8 shows the correlated response in milk yield during the 7 generations of selection for SCC. In all generations except generation 5, line LSCC exceeded in production both lines HSCC and CSCC. In generation 5, lines HSCC and LSCC were about equal in milk yield (25.32 vs 25.27 gms). Selection pressure for SCC applied in both selected lines, produced a negative correlated response in yield such that the original difference between selected lines in yield (1.39 gms) was more than doubled (3.08 gms) on the average by generation 7. The average correlated responses per generation, after adjusting for environmental effects, were small in both lines (0.067 ± 0.161 gms in line LSCC and -0.021 ± 0.185 gms in line HSCC) (Figure 8b). Another estimate of the average correlated response in yield per generation was the regression of the deviation HSCC-LSCC milk yield on generation number. This regression was small and negative (-0.088 ± 0.198). In addition, Figure 9 reveals that the trend of environmental effects on milk yield was negative.

The genetic regression measuring correlated response in milk yield per unit direct response in milk SCC was computed as follows. Pooled deviations of the selected lines from the control line (HSCC-CSCC and LSCC-CSCC) in milk yield were regressed on corresponding deviations for SCC. This regression was -0.162 ± 1.218 (Table 14).

Overall, these regressions indicate a small correlated response in milk yield opposite in direction from the direct response for milk SCC. This negative genetic relationship is consistent with the negative phenotypic correlation (-0.01) reported earlier between milk SCC and yield and consistent also with results reported in dairy cattle on the phenotypic relationship between the two traits (34,
Table 13. Milk yield of lines HSCC, CSCC and LSCC.

<table>
<thead>
<tr>
<th>Generation</th>
<th>HSCC</th>
<th></th>
<th></th>
<th>CSCC</th>
<th></th>
<th></th>
<th>LSCC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>Mean</td>
<td>SD</td>
<td>No. of mice</td>
<td>Mean</td>
<td>SD</td>
<td>No. of mice</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>0</td>
<td>29</td>
<td>25.43</td>
<td>3.26</td>
<td>28</td>
<td>24.94</td>
<td>3.74</td>
<td>38</td>
<td>26.82</td>
<td>5.35</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>27.98</td>
<td>4.26</td>
<td>28</td>
<td>27.92</td>
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<td>40</td>
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1. Mean milk yield in grams.
Figure 8. (a) Correlated response in lactation milk yield (gms). (b) Fitted linear regression lines.
Figure 9. (a) Change over time in lactation milk yield (gms) of line CSCC. (b) Fitted regression line.
Table 14. Genetic regressions and SE’s of correlated trait’s (selected-control lines) on milk SCC (selected-control lines)*.

<table>
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<tr>
<th>Trait</th>
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<th>SE</th>
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<td>Milk Yield</td>
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<tr>
<td>Blood SCC</td>
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<td>0.418</td>
</tr>
<tr>
<td>% Phag. Cells</td>
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<td>5.121</td>
</tr>
<tr>
<td>Resp. to End. Chall.</td>
<td>-3.201</td>
<td>1.396</td>
</tr>
<tr>
<td>% successful matings</td>
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<td>0.076</td>
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<tr>
<td>Number Born</td>
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<tr>
<td>% survival to weaning</td>
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<td>0.011</td>
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</table>

* Data combined for high and low lines; n = 8 (generations).
1. Lactation milk yield.
2. Log$_2$ of total leukocytes in blood.
3. Percentage of phagocytic cells (PMN and monocytes) in blood.
4. Response to E. coli endotoxin challenge (page 52).
5. Percentage of dams littering.
6. Number of young born (alive) in a litter.
7. Percentage of young (in a litter) surviving to weaning.
76). This conclusion could be justified based on the well known detrimental effect of elevated milk SCC on the secretory epithelium of the mammary gland (156).
TOTAL LEUKOCYTES IN BLOOD

Generation means and standard deviations for total cell counts in blood (expressed in the logarithmic and arithmetic scales) of the two selected lines (HSCC and LSCC) as well as of the control line (CSCC) are in Table 15. In addition, Figure 10 shows the correlated response in total cell counts in blood for 6 (of the 7) generations of selection for SCC, since blood SCC determinations were initiated in generation 2. In all generations mean blood SCC of line HSCC exceeded mean blood SCC of line LSCC. However, mean blood SCC of the control line exceeded mean blood SCC of one or both selected lines in all generations except generation 6. This fact, as well as the lack of divergence in blood SCC (Figure 10) for the two selected lines after eliminating environmental trends, indicated a lack of correlated response in blood SCC. The average correlated responses per generation, after adjusting for environmental effects were very small, positive and non-significant (P > .46) in both lines (0.019 ± 0.063 log units for line HSCC and 0.048 ± 0.060 units for line LSCC) (Figure 10b). The regression of the deviation HSCC-LSCC blood SCC on generation was also small and non-significant (P > .53), but negative (-0.029 ± 0.044) indicating that the original difference in blood counts between the selected lines decreased during the course of selection. In addition, Figure 11 reveals a very small negative environmental trend in blood SCC (line CSCC).

The genetic regression (regression of the pooled deviations of the selected lines from the control line in blood counts on the pooled deviations of the selected lines from the control line in SCC) was -0.087 ± 0.418 (Table 14 page 91). This regression was non-significant (P > .84) and suggested little change in blood SCC by selecting for milk SCC. This is consistent with results reported earlier on the phenotypic correlation between blood SCC and milk SCC (-0.09) and indicates that the two traits are probably not controlled by the same genes.

Total blood leukocytic counts have been used as a primary selection trait in two studies, both using the laboratory mouse as a model. In the first study Chai (28) selected for high (HLC) and low (LLC) total blood leukocyte counts. At eleven generations of selection, the mean leukocyte count of HLC was about three times that of LLC (about 13 vs 5 million cells/ml). Correlated responses
<table>
<thead>
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</table>

*No. of cells/mm³ (anti-log of mean log₂ leuk. no.).
Figure 10. (a) Correlated response in total leukocyte count in blood ($\log_2$). (b) Fitted linear regression lines.

Results and Discussion
Figure 11. (a) Change over time in total leukocyte count in blood ($\log_2$) in line CSCC. (b) Fitted regression line.
in numbers of various cell types (PMN, eosinophils, lymphocytes and monocytes) were proportional to their individual percentages of the total counts. In the second study Weir and Schlager (176) also selected two lines of mice for high (LCH) and low (LCL) blood cell counts. At generation 13 of selection the divergence between selected lines in blood SCC was maximum, with line LCH producing a mean approximately 4x the mean of line LCL (17.5 vs 4 million cells/ml). These two studies showed that blood SCC respond to selection relatively early (after 3-4 generations) and with a moderate intensity of response (realized heritability of about .17 (176)). However, results of the present study suggest that milk SCC and blood SCC are rather independent (genetically) traits.
PERCENTAGE OF PHAGOCYTIC CELLS

Generation means and standard deviations for percentage phagocytic cells (PMN and monocytes) in blood of lines HSCC, CSCC and LSCC are in Table 16. Also, Figure 12 shows the correlated response in percentage phagocytic cells during 7 generations of selection for milk SCC. There were large fluctuations in generation means of both selected lines with no consistent change in percent phagocytic cells in either line. The average correlated response per generation, after adjusting for environmental effects, was $0.353 \pm 0.819$ in line HSCC and $0.682 \pm 0.693$ in line LSCC (Figure 12b). Another estimate of the average correlated response per generation, the regression of the deviation HSCC-LSCC percent phagocytic cells on generation number, was $-0.329 \pm 0.657$ indicating a decrease of the original difference between the selected lines in percent phagocytic cells during the course of selection for milk SCC. In addition, Figure 13 reveals a negative environmental trend in percent phagocytic cells.

The genetic regression of percent phagocytic cells on milk SCC (after deviating the selected from the control lines), was estimated as $-3.492 \pm 5.121$ (Table 14 page 91). This negative genetic regression was non-significant ($P > .51$) and agrees with the negative phenotypic correlation ($-0.06$) reported earlier (page 73). However, the non-significant genetic regression, the absence of any trend in percent phagocytic cells and the failure of lines to diverge suggest that percentage phagocytic cells and milk SCC are independent (genetically) traits.

Percentage of phagocytic cells in blood has been studied as a correlated trait in the two studies (28, 176) discussed in the previous section. In both studies the primary selection trait was total blood cell counts. In the study by Weir and Schlager (176) after 20 generations of selection for high (LCH) and low (LCL) blood cell counts, the major portion of the difference in leukocyte count between the two selected lines, was accounted for by lymphocytes (the most frequent class of leukocytes in mice), although LCH mice had more cells of all categories. In LCL mice the number of PMN and eosinophils in blood increased under downward selection pressure for blood SCC. In the study by Chai (28), differential counts were influenced by selection for total blood counts.
<table>
<thead>
<tr>
<th>Generation</th>
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<th>SD</th>
<th>CSCC No. of mice</th>
<th>Mean&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SD</th>
<th>LSCC No. of mice</th>
<th>Mean&lt;sup&gt;1&lt;/sup&gt;</th>
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<td>24.09</td>
<td>10.23</td>
</tr>
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</table>

1. Mean percentage of phagocytic cells (% of total leukocytes in blood).
Figure 12. (a) Correlated response in percentage of phagocytic cells in blood. (b) Fitted linear regression lines.
Results and Discussion

Figure 13. (a) Change over time in percentage of phagocytic cells in blood in line CSCC. (b) Fitted regression line.
In fact, lymphocyte percentage decreased and PMN percentage increased in the LLC line, whereas in the HLC line the reverse was true.
RESPONSE TO CHALLENGE WITH ESCHERICIA COLI ENDOTOXIN

Generation means and standard deviations for response to challenge with E. coli endotoxin (expressed in the logarithmic and arithmetic scales) of lines HSCC, CSCC and LSCC are in Table 17. In addition, Figure 14 shows the correlated response in response to challenge for 5 (of the 7) generations of selection for milk SCC. Randomly chosen mice of the 3 lines were challenged with endotoxin, beginning in generation 3. In generations 3, 4 and 5 line HSCC yielded a higher response than either line LSCC or CSCC. However, in generations 6 and 7 the pattern was reversed with line LSCC yielding higher response than either line HSCC or CSCC. In fact, by generation 7, mice from line LSCC had increased, on average, their milk SCC (after an intramammary injection with endotoxin) more than 2x as compared with mice from line HSCC and 4x as compared with CSCC mice. The average correlated response per generation, after adjusting for environmental effects was -0.104 ± 0.128 in line HSCC and 0.647 ± 0.355 in line LSCC (Figure 14b). The regression of the deviation HSCC-LSCC response to challenge on generation was -0.751 ± 0.316 and nearly significant (P < .10). In addition, Figure 15 reveals a negative environmental trend in response to challenge (line CSCC).

The genetic regression of response to challenge on milk SCC (pooled over lines) was -3.201 ± 1.396 (Table 14 page 91). This regression was nearly significant (P < .06) and indicated a negative genetic relationship between milk SCC and response to challenge. This is consistent with the negative phenotypic correlation (-0.14) between the two traits reported earlier (Table 7) and indicates that selection for low milk SCC results in an increase of the ability of the individual mouse to elevate milk SCC after an injection with E. coli endotoxin.

It is difficult to present a physiologic explanation for these results. The difficulty (101) arises from the fact that the role of leukocytes in general (mainly PMN) in phagocytosis (in response to bacterial products as well) is less than perfectly understood. Many authors (72, 115, 124, 139) have reported on the role of PMN in phagocytosis against mastitis causing bacteria. Miller and Schultzze (101) after an extensive review concluded that more research is needed on the physiologic aspects
Table 17. Response to endotoxin challenge, of lines HSCC, CSCC and LSCC.

<table>
<thead>
<tr>
<th>Generation</th>
<th>HSCC</th>
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* Mean of the difference challenged-control glands milk SCC expressed as no. of cells/mm³ (anti-log of mean log₂ leuk. no.).
Figure 14. (a) Correlated response in response to E. coli endotoxin challenge (SCC in milk of challenged glands). (b) Fitted linear regression lines.
Figure 15. (a) Change over time in response to E. coli endotoxin challenge (SCC in milk of challenged glands) in line CSCC. (b) Fitted linear regression line.
of response to intramammary infections. In addition, response to live bacteria and response to bacterial products might be less than perfectly correlated measures of response. Weir and Schlager (176) reported results which may be related to results of this study. In their experiment, mice of the high (LCH) and low (LCL) blood SCC lines, which at generation 13 differed by 13.5 million cells/ml of blood, were injected with live or heat-killed Salmonella typhimurium. There were no significant differences between lines in resistance to a standard dose of live organisms, to the toxic effects of massive doses of killed organisms, or to reinoculation with live organisms after immunization. These results suggest that changes in peripheral leukocyte number per se do not enhance or reduce resistance. However, in the present study, differences between lines in blood SCC were small with no tendency to change with selection for milk SCC. In addition, response was measured with respect to the mammary gland only. Results of the present study therefore do not seem contradictory to those of Weir and Schlager (176) in indicating that low milk SCC might be advantageous for the individual with regard to responding to bacterial endotoxin infections of the mammary gland. However, the physiological basis of this result is unclear and more data are needed to confirm this conclusion.
**FITNESS**

Somatic cells with phagocytic ability represent one aspect of an animals' defence mechanism against certain diseases. It therefore is possible that selection to alter SCC may result in correlated changes in general fitness of the animal. To examine this possibility, correlated changes in three measures of fitness were monitored over the course of selection. Measures included percentage of females exposed to males which eventually littered, number of young born alive per litter, and percentage of young in a litter (including fostered young) which survived to weaning.

**Percentage of females littering**

Generation means and standard deviations for percentage of females littering in lines HSCC, CSCC and LSCC are in Table 18. In addition, Figure 16 shows the correlated response in percentage littering for 7 generations of selection for milk SCC. There were sizable fluctuations in mean percentage littering in both selected lines (Figure 16) as well as in line CSCC (Figure 17). However, the percentage of dams littering was high (> 70%) in all lines and generations. The average correlated response per generation, after adjusting for environmental effects was \(-0.014\pm 0.007\) in line LSCC and \(-0.005\pm 0.013\) in line HSCC (Figure 16b). The regression of the deviation HSCC-LSCC percent littering on generation was \(0.009\pm 0.011\) and non-significant (\(P > .44\)). In addition, Figure 17 reveals a small negative environmental trend in percentage littering (line CSCC). The genetic regression of percentage littering on milk SCC was \(0.073\pm 0.076\) (Table 14 page 91). These extremely small regressions indicate little, if any, change in the rate of successful mating and gestation due to selection for either increased or decreased SCC in milk.
Table 18. Percentage of females littering, of lines HSCC, CSCC and LSCC.

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1. Mean percentage of successful matings.
Figure 16. (a) Correlated response in percentage littering. (b) Fitted linear regression lines.
Figure 17. (a) Change over time in percentage littering in line CSCC. (b) Fitted linear regression line.
Number of young born alive

Generation means and standard deviations for number of young born alive per litter in lines HSCC, CSCC and LSCC are in Table 19. In addition, Figure 18 shows correlated response in number born for 7 generations of selection for milk SCC. Mean number young born in all lines varied considerably across generations (Figures 18, 19) but with no time trend in any line. The average correlated response per generation, after adjusting for environmental effects was -0.040 ± 0.090 in line LSCC and -0.062 ± 0.069 in line HSCC (Figure 18b). The regression of the deviation HSCC-LSCC number born on generation was -0.022 ± 0.065. In addition, Figure 19 reveals a small positive environmental trend in number born (line CSCC). The genetic regression of number of young born on milk SCC was -0.442 ± 0.566 (Table 14 page 91). These small regressions on generation number and on direct response indicate no correlated change in number born alive due to selection for increased or decreased SCC in milk.
<table>
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1. Mean number of young born alive per dam.
Figure 18. (a) Correlated response in number of young born. (b) Fitted linear regression lines.
Figure 19. (a) Change over time in number of young born in line CSCC. (b) Fitted linear regression line.
Percentage of young surviving to weaning

Means and standard deviations for percentage of young surviving to weaning by line-generation are in Table 20. Also, Figure 20 shows the correlated response in percentage survival during 7 generations of selection for milk SCC. Means of percent survival in all lines varied only slightly across generations and without time trend (Figure 20). In all lines and generations the percentage survival was very high (> 97%). Average correlated responses per generation, after adjusting for environmental effects, were 0.0023 ± 0.0011 in line LSCC and 0.0021 ± 0.0014 in line HSCC (Figure 20b). The regression of the deviation HSCC-LSCC percent survival on generation was -0.0002 ± 0.0007. In addition, Figure 21 reveals a small negative environmental trend in percent survival.

The genetic regression of percent survival on milk SCC was 0.005 ± 0.011 (Table 14 page 91). This very small, non-significant (P > .64) genetic regression indicated that selection for milk SCC did not affect percentage of young surviving to weaning.

Monitored changes in percentage females littering, number born alive, and percentage surviving to weaning indicated that neither selection for increased nor decreased milk SCC affected fitness. This is consistent with the expectation based on results reported earlier on the phenotypic relationship between milk SCC and fitness (regressions of fitness traits on milk SCC were no different than zero) as well as with the very low inbreeding coefficients found (0.005 in the selected and 0.007 in the control lines). Similar results were reported by Weir and Schlager (176) who selected for high and low blood SCC in mice. Although the system of mating they used in the selected lines was exclusively based on matings between full-sibs, no significant differences between lines were found in number born or in number of mice weaned in a litter. However, in a more recent study Chai (28), found significant differences in reproductive performance between two lines, one selected for high blood counts (HLC) and another for low counts (LLC). Line LLC had much higher sterility (33 %) than the HLC (7 %) and the control (9 %) lines. In addition, line LLC produced and weaned on the average 0.3 mice less and had 0.13 more still births per litter than the HLC line. The author suggested that low blood counts may had been the reason for reduced fitness in the early part of
Table 20. Percentage of young surviving to weaning of lines HSCC, CSCC and LSCC.

<table>
<thead>
<tr>
<th>Generation</th>
<th>HSCC</th>
<th></th>
<th></th>
<th>CSCC</th>
<th></th>
<th></th>
<th>LSCC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of dams</td>
<td>Meanⁱ</td>
<td>SD</td>
<td>No. of dams</td>
<td>Meanⁱ</td>
<td>SD</td>
<td>No. of dams</td>
<td>Meanⁱ</td>
<td>SD</td>
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<tr>
<td>0</td>
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<td>0.99</td>
<td>0.03</td>
<td>32</td>
<td>1.00</td>
<td>0.02</td>
<td>42</td>
<td>1.00</td>
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<td>1.00</td>
<td>0.00</td>
<td>46</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>1.00</td>
<td>0.00</td>
<td>31</td>
<td>1.00</td>
<td>0.02</td>
<td>43</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>1.00</td>
<td>0.00</td>
<td>33</td>
<td>0.99</td>
<td>0.03</td>
<td>43</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>0.99</td>
<td>0.04</td>
<td>30</td>
<td>0.99</td>
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<tr>
<td>5</td>
<td>45</td>
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<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>0.99</td>
<td>0.02</td>
<td>28</td>
<td>1.00</td>
<td>0.00</td>
<td>48</td>
<td>1.00</td>
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</tr>
<tr>
<td>7</td>
<td>27</td>
<td>1.00</td>
<td>0.00</td>
<td>31</td>
<td>0.98</td>
<td>0.05</td>
<td>36</td>
<td>1.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1. Mean percentage of young per dam, surviving to weaning.
Figure 20. (a) Correlated response in percentage of young surviving to weaning. (b) Fitted linear regression lines.
Figure 21. (a) Change over time in percentage of young surviving to weaning in line CSCC. (b) Fitted linear regression line.
the study (up to generation 7). Increase in the rate of inbreeding starting from generation 8 was an additional factor reducing fitness in line LLC.
Conclusions

Results of this experiment indicate that the laboratory mouse is a useful animal model in the study of relationships between milk SCC, milk yield, total and differential blood cell counts, and intramammary infections in dairy cows. This is so mainly because of the short generation interval in mice (3 months) as compared to cows, as well as because the methods developed in this study to obtain data for milk SCC in mice lead to rapid and accurate cell determinations. In addition, simulation of intramammary infections using E. coli endotoxin has proved to be as good a stimulant for elevating milk SCC in mice as in other species (cows, guinea pigs etc.)

Distribution of milk SCC in mice (reported for the first time) is similar to that of cows to a constant multiplier. However, the large increase in SCC during late lactation of the mouse may suggest early and midlactation counts are preferred for modeling relationships in dairy cattle. Results also indicate a within lactation repeatability of SCC similar to that reported in cattle.

The lactation curve determined in this study was similar to that in cattle but different than that reported in previous mouse studies (maximum daily milk yield in early lactation). However, maximum daily yield and average lactation yield determined in this study were intermediate to figures reported in other mouse studies.
The phenotypic regression of lactation milk yield on lactation milk SCC was negative indicating that dams with higher SCC in milk produce less milk. This is possibly due to the well known detrimental effects of SCC on the secretory epithelium of the mammary gland. However, it should be pointed out that this negative effect of SCC on yield becomes obvious at higher levels of cell counts than in dairy cows. Small positive correlations found between milk yield and number young born as well as between yield and percentage survival indicate that dams producing larger litters also had higher lactation yield and tended to wean more young than dams producing smaller litters (despite the fact that litters were standardized).

Very small negative phenotypic correlations between milk SCC and blood SCC as well as between milk SCC and percentage phagocytic cells were found, suggesting that these three traits are not-related, phenotypically. In addition, a small but significant negative phenotypic correlation between milk SCC and response to endotoxin challenge was found, indicating that dams with inherently higher milk SCC responded less to E. coli endotoxin challenge than dams with lower milk SCC. A small positive phenotypic correlation between response to challenge and percentage phagocytic cells in blood suggested that dams with higher percentages of phagocytic cells tended to have slightly greater response to endotoxin challenge than dams with lower percentages. Finally, phenotypic correlations between milk SCC and three fitness traits (% littering, number born and % survival) were negligible suggesting that milk SCC and fitness are independent traits.

No major mastitis causing pathogens in cattle such as Strep. species (except Strep. fecalis) and/or coliforms were detected in mice involved in the study. In addition, bacteria detected (Bacillus sp., Corynebacterium sp. etc.) did not cause any serious mastitis infections and/or increase in milk SCC.

The desirability of reducing mastitis incidence in dairy cattle through selection for low milk SCC has been expressed in other studies. However, the possibility of reducing the ability of future generations to respond efficiently to mammary infections has made other researchers skeptical about selecting for low milk SCC. Results in the present study suggest first that rate of response to selection for high or low milk SCC, at least as determined by the animal model used, is moderate.

Conclusions
A considerable difference in milk SCC between two lines of mice (HSCC and LSCC) was created in seven generations of full-sib family selection (lines started separating from generation 3). The response to selection was symmetrical and in the expected direction in both lines.

The small negative genetic regression (pooled regression of deviations of selected lines from control line for the secondary trait, on deviations of selected lines from control line for the primary trait) of milk yield on milk SCC suggested a small correlated response in milk yield opposite in direction from the direct response for milk SCC. Very small and inconsistent negative genetic regressions of blood SCC and percentage phagocytic cells on milk SCC were found, suggesting that these traits are independent (genetically) of milk SCC. The significance of this is that selection in either direction for milk SCC will be expected to produce little, if any, change in circulating leukocytic pools and percentage of phagocytic cells. This suggests that no change will be brought about in the systemic resistance of animals as a result of selection for milk SCC.

In early generations, line HSCC yielded a higher response to endotoxin infusions, than either line LSCC or CSCC. However, in the late part of the experiment the pattern was reversed with line LSCC yielding higher response than either line HSCC or CSCC. A negative genetic regression of response to challenge on milk SCC was found indicating that selection for low milk SCC results in an increase of the ability of the individual mouse to elevate milk SCC after an injection with E. coli endotoxin. The physiological basis of this result is not known and more data are needed to confirm this conclusion. In addition, if we accept that response to live bacteria and response to bacterial products (used in this study) might be less than perfectly correlated measures of response, it would be necessary to observe the behavior of the divergent lines to intramammary inoculations with live bacteria, before we accept the conclusion that low milk SCC are advantageous for an animal. Finally, results on fitness traits indicate no correlated change in percentage of females littering, number of young born alive or percentage of young surviving to weaning due to selection for increased or decreased SCC in milk.

Conclusions
It should be emphasized that the more conventional methods of mastitis control, including hygiene and antibiotic therapy, will remain the cornerstones of any well designed mastitis control program. They will remain the key methods of preventing new infections from developing and eradicating existing mastitis cases. However, selection should be a valuable addition to a mastitis control program, because it should gradually improve the overall mastitis health status of the herds in which it is applied. It may be that a measure of SCC could be best combined with measures of other economically important traits in a selection index designed to maximize a cow’s lifetime profitability, rather than selecting for increased genetic resistance to mastitis.


Bibliography


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Appendix A. Effect of Stage of Lactation on Milk SCC.
Table 21. Effect of stage of lactation on milk SCC\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS\textsuperscript{2}</th>
<th>F\textsuperscript{3}</th>
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</thead>
<tbody>
<tr>
<td>Line</td>
<td>2</td>
<td>5.473</td>
<td>1.67</td>
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<tr>
<td>Mating group</td>
<td>2</td>
<td>33.076</td>
<td>10.07**</td>
</tr>
<tr>
<td>Day\textsuperscript{4}</td>
<td>2</td>
<td>900.806</td>
<td>274.35**</td>
</tr>
<tr>
<td>Milk\textsuperscript{5}</td>
<td>1</td>
<td>57.744</td>
<td>17.59**</td>
</tr>
<tr>
<td>Milksq\textsuperscript{6}</td>
<td>1</td>
<td>13.404</td>
<td>4.08*</td>
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<tr>
<td>Error</td>
<td>1154</td>
<td>3.283</td>
<td></td>
</tr>
</tbody>
</table>

1. The correction factors developed from this ANOVA were from Least Square Means computed for each of the three days 9, 14, and 18. These factors were 1.02 for day 9 and .86 for day 18.
2. Mean Square.
3. Effects with * were significant at level .05. Those with ** were significant at level .01.
4. Lactation day 9, 14 or 18.
5. Effect of milk yield on day 8 or 13 or 17.
6. Effect of the quadratic milk yield of day 8 or 13 or 17.
Appendix B. Repeatability of log₂ SCC.
Table 22. Repeatability of log₂ SCC¹.

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS²</th>
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</thead>
<tbody>
<tr>
<td>Line</td>
<td>2</td>
<td>9.109</td>
</tr>
<tr>
<td>Mating group</td>
<td>2</td>
<td>32.129</td>
</tr>
<tr>
<td>Dam(Line*Mating group)</td>
<td>467</td>
<td>3.812</td>
</tr>
<tr>
<td>Error</td>
<td>689</td>
<td>2.697</td>
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</tbody>
</table>

1. The K value for the effect of dam computed in this ANOVA was 2.458.
2. Mean Square.
Appendix C. Effect of postinjection (with E. coli endotoxin) time of SCC determination on level of SCC.
Table 23. Effect of postinjection (with E. coli endotoxin) time of SCC determination on level of SCC (n = 12 mice).

<table>
<thead>
<tr>
<th>Time of SCC determination</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before injection</td>
<td>959.17</td>
<td>174.27</td>
</tr>
<tr>
<td>8 h postinjection</td>
<td>4722.50</td>
<td>1143.22</td>
</tr>
<tr>
<td>24 h &quot;</td>
<td>52919.17</td>
<td>8009.56</td>
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<tr>
<td>48 h &quot;</td>
<td>17334.17</td>
<td>2253.38</td>
</tr>
<tr>
<td>72 h &quot;</td>
<td>1208.33</td>
<td>178.38</td>
</tr>
</tbody>
</table>

1. SCC expressed in cells/mm³

2. Three glands per mouse injected with 50 µl per gland of 100 µg/ml E. coli endotoxin.

3. Means of SCC at 24 and 48 h postinjection were different than the mean SCC before injection (P < .01). Means of SCC at 8 and 72 h postinjection were not different than the mean SCC before injection (P > .40).
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The two page vita has been removed from the scanned document. Page 2 of 2