Selection for Milk Somatic Cell Count in Laboratory Mice

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(ABSTRACT)

A bidirectional selection experiment for high and low somatic cell count (SCC) was conducted over 14 generations with two selected lines (high line = HSCC, low line = LSCC) of mice. Seven secondary traits (milk yield, total white blood cell count, percentage of phagocytic cells in blood, endotoxin challenge response, percentage of females littering, number of young born alive, and percentage of young surviving to weaning) were measured to examine correlated responses to selection for SCC.

Average response per generation for $\log_2$ SCC was small in both selected lines (HSCC = .0678 ± .0341, LSCC = .0384 ± .0390, $P > .05$). There was little per generation divergence between the selected lines (.0294 ± .0178, $P > .05$). Genetic and phenotypic selection differentials indicated that selection procedures did select the more extreme individuals for SCC, even though response to selection was poor.

Phenotypic correlations among SCC and the seven secondary traits were generally small, and near zero. Correlation coefficients ranged from -.17 to .17. Milk yield was negatively correlated with SCC (-.07, $P < .05$). The correlation between endotoxin challenge response and SCC was also negative (-.17, $P < .05$).

Components of genetic variance for SCC were estimated to explain the lack of selection response. Covariances between daughter and dam, and among full sibs were negative (-.1180 and -.0362, respectively). Analysis for offspring and maternal components for SCC yielded a negative estimate for the covariance between additive effects for the offspring and maternal components (-.1781).

No biological explanation can be offered for its existence. Heritability from this same analysis was .05.

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Introduction

Milk somatic cell count is a measure of the health status of the mammary gland. During infection of the mammary gland (mastitis), large numbers of leukocytes infiltrate the gland and function to eliminate bacteria, consequently increasing somatic cell counts (SCC) in milk. In this way SCC is an indirect measure of mastitis and is especially useful in identifying subclinical mastitis.

During recent years dairymen have become increasingly aware of the economic costs associated with mastitis. Estimates of annual loss in the U.S. range from .5 to 2.0 billion dollars (5, 25, 41, 53). The greatest portion of the losses incurred by mastitis is attributed subclinical mastitis (65 to 70%) (28).

The positive genetic relationship between mastitis and milk yield is cause for additional concern. Current genetic gain for milk yield accounts for 50 to 60 percent of the improvement in annual yield per cow (62). Shook (62) reported results from simulation studies concerning the effects of selection for milk yield on mastitis. Assuming a genetic correlation of .3 between yield and mastitis, it was found that selection for milk yield was accompanied by an increase of .4% in mastitis incidence and .02 mastitis cases per year. Studies such as these indicate that genetic improvement for milk yield is causing a slow increase in mastitis incidence. From an economic standpoint the increased cost

of mastitis is justifiable because it is more than offset by the increased value of milk sold (62). However, from a health standpoint the increase in mastitis incidence is cause for concern.

Current hygiene and management practices have effectively reduced rates of mastitis incidence caused by contagious pathogens, however, environmental mastitis is more difficult to bring under control. With the increase expected in mastitis incidence as selection for milk yield continues, focus on mastitis prevention research is imperative. One path to follow in mastitis research is to look for ways to improve the inherent physiological defense mechanisms of the cow. Leukocytes which infiltrate the mammary gland during infection are one such defense mechanism. In recent years there has been discussion of the possibility of increasing genetic resistance to mastitis through selection based on SCC. The purpose of this study was to examine the feasibility of selection for SCC using the laboratory mouse as a model for the dairy cow. The objectives of this study were as follows:

1) To develop lines of mice genetically divergent for milk somatic cell concentration through single trait selection.

2) To estimate correlated responses to selection for SCC and characterize the lines with respect to:

- milk yield,
- blood leukocyte pools and their composition,
- ability to mobilize leukocytes in response to endotoxin challenge of the mammary gland, and
- general fitness and reproductive performance.

Literature Review

Economic Importance of Mastitis

Dairymen in the United States incur large economic losses which are attributable to mastitis. In 1970 Janzen (25) estimated annual loss to be 400 to 500 million dollars. Nielsen (53) and Blosser (5) reported estimates of 600 to 800 million and 1.3 billion dollars, respectively, in 1976. Blosser (5) used survey responses from 33 states representing 86% of the U.S. dairy cow population. A more recent study by Miller (41) placed total economic loss for the U.S. at $2 billion, annually.

Losses attributed to mastitis can be broken down into the following categories: 1) reduced milk production resulting from clinical and subclinical infections, 2) milk discarded because of antibiotic residues or abnormal composition, 3) veterinary fees, 4) drug costs, 5) cost of increased labor to care for mastitic cows, 6) increased replacement cost for cows culled because of mastitis, and 7) decreased sale value of animals sold for dairy purposes. Reduced milk production accounts for the largest proportion of the losses incurred by mastitis (5, 15).

Estimates of reduced or "lost" milk production resulting from mastitis can be found expressed on a per infection, per quarter, per lactation or per year basis and are derived from clinical cases,

California Mastitis Test (CMT) scores, results of implementing mastitis control programs, and milk somatic cell counts (SCC). A study involving 1243 cows in 10 commercial herds over 305 days of lactation, reported an average milk loss of 6.0, 10.0, 16.0, and 24.5 percent associated with CMT scores of trace, 1, 2, and 3, respectively (21). Another study (20) compared opposite quarter milkings of 763 cows in 30 herds and found losses of 9.0, 19.5, 31.8, and 43.4 percent/quarter/day associated with CMT scores of trace, 1, 2, and 3, respectively. A 1977 review cited a Cornell study which estimated the average loss from a single infected quarter to be 1700 lb/year (15). This was comparable to a 1972 study which reported an average loss of 740 kg/cow/year from each infection (50).

Janzen (25) and Dobbins (15) both reported a drop in cost per cow per year of 2/3, two years after the implementation of a mastitis control program. Blosser's survey study (5) reported a dollar value of 897 million for lost milk production in the U.S., annually. This figure is 69.3% of the annual total U.S. economic losses resulting from mastitis.

The need for an objective measure of mastitis and losses in milk production resulting from mastitis (both subclinical and clinical) have led to the use of milk SCC's in research. From a quarter comparison study involving 874 quarters, Ward and Schultz (72) found that a quadratic regression indicated losses of 15% and more than 25% from quarters with SCC's of 2 and 4 million cells/ml, respectively. A 3 year study covering 108 lactations reported that a SCC over 500,000 cells/ml reduced total lactation milk production by more than 20 percent (40). Another study by Schultz (60) found that cows with an SCC averaging less than 500,000 cells/ml produced on average 572 kg more than cows with an average lactation SCC above 1 million cells/ml. Finally, a review by King (31) cited a 1971 Milk Marketing Board study in Great Britain which attributed losses of 164, 289, 661 and 770 kg/cow/year to SCC ranges of 250,000 to 499,000, 500,000 to 749,000, 750,000 to 949,000 and 1,000,000 and over cells/ml.

Aside from lost milk production, another source of loss associated with mastitis is incurred from milk discarded due to treatment of the cow with antibiotics. Natzke (48) estimated this loss to be

27 kg/day for 5 days for each clinical case and 1 to 1.5 clinical cases/cow/year in herds without teat dip and dry cow therapy programs. This would be 135 to 203 kg/cow/year discarded as a result of mastitis. From survey responses Blosser (5) estimated the loss from milk discarded due to antibiotic treatment to be $12.88/cow/year.

Veterinary service costs incurred by mastitis were estimated to be $.62/cow/year ($125/year for a 200 cow herd) by Natzke in 1976 (48). This estimate was for cows not involved in teat dip and dry cow therapy programs. The same author estimated antibiotic costs for mastitis treatment to be $2.50/cow/year ($500/year for a 200 cow herd) (48). This again was for cows not involved in teat dip and dry cow therapy programs. Blosser's (5) study reported the annual per cow losses associated with veterinary fees and drug costs to be $1.97 and $3.86, respectively.

The increase in labor required to care for mastitic cows was estimated by Natzke (48) to average 250 hours/year for a 200 cow herd and cost $5.00 per hour. Stated another way this is $6.25/cow/year. From Blosser's study (5) the cost estimate for increased labor was $2.28/cow/year.

The last category of losses to be discussed is the impact of mastitis on sale value and replacement cost. Estimates of the costs incurred by these factors are not prevalent in the literature. Those from Blosser's study (5) indicate an annual per cow loss of $5.72 and $9.32 for decreased sale value and increased replacement cost, respectively. The total per cow annual cost associated with mastitis was $117.35 from this same study.

With regard to SCC, a study conducted at the University of Wisconsin measured clinical treatment costs associated with mastitis and economic benefit that can be expected from reducing SCC (62). Treatment costs included drug costs, veterinary fees, discarded milk and increased labor. Costs were expressed as the amount of milk containing 3.5% fat and 3.2% protein at the market price equivalent to the treatment cost. It was found that reducing the lactation geometric mean SCC by 50% for an older cow lowered average treatment cost per lactation by the equivalent of the market value of 400 lb milk (62).

Etiology and Control of Mastitis

Mastitis is an inflammation within the mammary gland. Schalm et al. (57) refer to mastitis as a "disease complex" because of its different causes, different degrees of intensity, and its variation in duration and residual effects. Any type of injury to the internal tissues of the mammary gland will lead to mastitis. However, mastitis most commonly begins as a result of penetration by pathogenic bacteria through the teat canal into the interior of the gland. If the internal environment of the gland is conducive to bacterial multiplication, the resulting products of bacterial growth and metabolism will irritate the internal tissues leading to an inflammatory response, or mastits (57). The clinical and subclinical signs associated with mastitis (swelling, heat, redness, pain, disturbed function, fever, depresion, changes in milk composition and increases in SCC) are an expression of a defense mechanism whose purpose is to destroy the invading pathogen and allow for repair and return of the gland to normalcy (57).

Bacteria capable of causing mastitis are commonly widespread in the environment of the cow. They present a constant threat to the mammary gland of the cow. In a report on environmental mastitis (infections caused by pathogens whose primary reservoir is the cow's environment), Smith et al. (66) summarized and discussed work related to the role of specific pathogens in mastitis and control methods employed against mastitis. They commented that generally a distinction is made between the contagious pathogens *Streptococcus agalactiae* and *Staphylococcus aureus*, and environmental pathogens. The primary reservoir of *Strep. agalactiae* and *Staph. aureus* is an infected quarter. Teat ends of infected quarters are exposed to these pathogens primarily during the milking process. Postmilking disinfection of teat ends greatly reduces new infection (66).

Exposure of teat ends to environmental pathogens occurs between milkings. Postmilking disinfection of teat ends is either less effective or ineffective at reducing the rate of new infection by environmental pathogens (66). Addtionally, with regard to environmental pathogens, those most frequently encountered include environmental streptococci (streptococci other then *Strep.*

agalactiae) and coliform bacteria. Species of environmental streptococci involved in mastitis include *Streptococcus uberis, Streptococcus bovis, Streptococcus faecalis, and Streptococcus dysgalactiae.* Coliform bacteria generally encountered include *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytocia, Enterobacter aerogenes* and species of *Citrobacter, Serratia* and *Proteus* (66).

Prior to the widespread use of teat dipping and dry cow therapy, studies indicated that 50% of all cows were infected in two or more quarters (66). Furthermore, 80 to 90 percent of these infections were caused by the contagious pathogens *Strep. agalactiae* and *Staph. aureus*. Only a small percentage of infected quarters involved environmental pathogens, and coliform bacteria were seldom isolated from more than 1 to 2 percent of the quarters (66).

Studies on practical hygiene procedures were begun in 1960 by researchers from the National Institute for Research in Dairying; Reading, England. From these studies and a later study done in conjunction with Cornell University came the basis for the current recommendations for the use of teat dip and dry cow therapy (16, 49, 65). Postmilking disinfection of teat ends reduces rates of new infection by effectively killing organisms on the teat end. Dry cow therapy shortens duration of infection by clearing existing infections at drying off and reduces rates of new infection occurring during the dry period. The combination of teat dip and dry cow therapy was found to reduce rates of infection to less than 8% of the quarters. However, while these two control methods are effective against contagious pathogens, their success is limited in controlling mastitis caused by environmental pathogens (16, 49, 65, 66). Environmental pathogens can be reduced through proper maintenance of housing and yards, but there appears to be no practical means of eliminating mastitis resulting from direct contact with sources of environmental pathogens.

The role of the development of electronic cell counting procedures in mastitis control is discussed in reviews by both Natzke and Dodd (49, 16). Persuading dairymen to take action against a disease which probably affected more than half their herd (prior to adoption of teat dip and dry cow therapy) but which could not be seen due to the frequency of its subclinical expression was a problem.

Electronic cell counting services by Dairy Herd Improvement laboratories provided a way to make dairymen aware of the importance of the level of subclinical infection in their herds.

The present control program of teat dip and dry cow therapy functions by reducing rates of new infection and shortening duration of infection. Areas for further research on mastitis control include improving the present control program and improving the natural defense mechanisms of the cow by decreasing susceptibility or increasing resistance to mastitis. As previously discussed the present control program has effectively reduced rates of infection by contagious pathogens. However, it's effectiveness against environmental pathogens is limited. The second option for future research, enhancing the natural defense mechanisms of the cow, may offer greater opportunity for controlling mastitis caused by environmental pathogens.

Defense Mechanisms of the Mammary Gland

The Teat Canal

Intramammary infection occurs when bacteria penetrate the teat canal, progress to the milk producing tissues and there multiply in numbers sufficient to cause inflammation. The teat canal with its physical and chemical antibacterial properties is the first defense barrier against mastitis. Two reviews by Nickerson (51,52) discuss the defense mechanisms of the teat canal. During the intermilking period the teat canal has been reported to be folded and occluded with keratin (52). Keratin is derived from epithelial cells lining the canal which continuously slough off into the lumen of the canal (57). The mesh-like character of keratin inhibits upward progress of bacteria into the gland (51). Furthermore, keratin contains lipids and proteins with bacteriocidal and bacteriostatic properties (51), and bacteria have been shown to adsorb to the lipids coating the cells which make up keratin (52).

It has been reported (51) that the removal of keratin decreases resistance to intramammary infection, however, following its replacement quarters were again found to be resistant. Microscopic examination has demonstrated that the keratin lining from susceptible quarters is thinner, less dense and detached from the epithelium when compared to resistant quarters. Additionally, resistant and susceptible quarters have been reported to differ in the acid composition of their keratin (51).

Cationic proteins have been isolated from keratin and shown to strongly inhibit growth of two major pathogens, *Strep. agalactiae* and *Staph. aureus* (51). In contrast, other researchers have noted that common udder pathogens can utilize teat canal lipids as a growth substrate (52). This can lead to colonization of the canal and in turn to the development of a teat canal infection. Teat canal infections occur in both lactating and nonlactating cows and may survive for up to three months (51).

Additional defense mechanisms of the teat canal include the sphincter muscle which surrounds the canal, the flushing action of fluid during the milking process and the tissues lining the canal (51). The sphincter muscle functions by maintaining tight closure of the canal and limiting bacteria to the teat orifice. Nickerson (51) reported that loss of muscle tonus may lead to increased susceptibility to intramammary infection, greater incidence of infection having been demonstrated in quarters with patent (leaky) canals.

Frequent flushing of fluid during the milking process removes bacteria colonizing the canal lining and teat orifice (51). Following milking the canal lumen remains dialated for up to two hours. This finding led to the suggestion that cows be fed immediately after milking to keep them on their feet and allow for constriction of the canal lumen and teat orifice (51).

Nickerson (51) cited a study undertaken to better understand the role which tissues lining the canal play in susceptibility and resistance to mastitis. Tissue thickness was found to increase across all stages of lactation, while a significant decrease in tissue thickness was found in infected quarters. Nickerson commented that decreased tissue thickness may render quarters more susceptible by

providing an inadequate physical barrier as well as a reduction in bacteriostatic substances. Lastly, Schalm et al. (57) attributed increasing infection with age patterns to a break down of the teat canal barrier with advancing age.

Phagocytosis

The second defense barrier of the mammary gland against invading bacteria is phagocytosis by white blood cells (leukocytes). Cells present in milk from uninfected glands consist of the following: neutrophils 0 to 11, lymphocytes 10 to 27, macrophages 66 to 68, epithelial cells (ductal) 0 to 7 and miscellaneous less than 1 percent (34). During infection leukocyte populations in milk change. A large influx of leukocytes enter mammary tissue via diapedesis. Their great motility and ability to deform and squeeze through spaces one third their size enable them to cross tissue barriers during diapedesis (24). Once in the mammary gland, phagocytic leukocytes (neutrophils and macrophages) ingest and kill bacteria intracellularly. During infection, neutrophils comprise the majority of the cells in milk, generally 90% or greater (24, 51, 52).

Speed of neutrophil mobilization and rapid recognition of bacteria contribute to phagocytic competence (42, 52). Speed of mobilization affects the total number of leukocytes available at the site of infection, while rapid recognition of bacteria is associated with individual cell competence (42). Milk presents a less than optimum environment for phagocytosis. The phagocytic capacity of cells in milk has been shown to be decreased in comparison to phagocytic cells isolated from the blood (42, 52). Decreased phagocytic capacity in milk is influenced by the lack of a suitable energy source, lack of opsonins, low oxygen levels and ingestion of milk fat and casein (42, 52).

Cows have been reported to vary in degree of neutrophil diapedesis, phagocytic activity and ability to kill intracellularly (52). Attempts have been made to enhance these natural defense mechanisms through the use of intramammary devices (IMD) and intracisternal beads (ICB). Both of these devices are used to stimulate a sterile inflammation with leukocytosis into the milk. Neutrophil

elevation induced by the IMD was found to prevent intramammary infection during experimental challenge studies (52). In a similar manner the ICB stimulated neutrophil infiltration of the distal teat tissues and enhanced resistance during early involution when the degree of leukocytosis is decreased (52).

Although phagocytosis is a defense mechanism, research by Capuco et al. (7) has raised some concern that the normal activity of phagocytosing neutrophils may have a detrimental effect on mammary secretory tissue. Mammary explants were treated with varying concentrations of intact, lysed or phagocytosing neutrophils. Measures of epithelial damage were greatest from those explants treated with phagocytosing neutrophils. However, even with epithelial damage, rates of milk protein and fatty acid synthesis were not affected. Release of hydrolytic enzymes during neutrophil degranulation is believed to be responsible for the damage inflicted on the secretory tissue (7).

As previously mentioned, mammary pathogens can utilize teat canal lipids as a growth substrate and colonize the teat canal (52). Nickerson (51) has indicated that teat canal colonization is a prelude to mastitis more often then is commonly realized. Quantitative and ultrastructural studies have found a preferential infiltration of neutrophils into the Furstenberg's rossette region during infection (51). Elevated numbers of neutrophils in teat tissue during the initial stages of infection may exert considerable influence on the progress of bacteria by destroying them before they reach the delicate secretory tissue. Any contribution to epithelial cell lysis by neutrophils en route to the teat cistern would be less harmful to teat tissue compared to secretory tissue (51). Whether or not neutrophil functions can be detrimental to secretory tissue, they play a key role in the defense of the mammary gland against infection. This has been demonstrated by the work of Schalm et al. (58) who reported an uncontrolled bacterial multiplication resulting from the absence of neutrophils during infection.

Immune Response

In contrast to the nonspecific nature of the phagocytic response, the immune response is highly specific. The functions of the immune response are to recognize, selectively eliminate and remember foreign invaders of the mammary gland (54). The immune response can be subdivided into humoral and cell mediated immunity (CMI) (42, 54, 69). Humoral immunity refers to the role of immunoglobulins (Ig) secreted by B-lymphocytes. Cell mediated immunity is based upon the presence and production of T-lymphocytes which are sensitive to foreign cells and molecules (42, 54, 69).

Humoral immiunity and CMI are not independent phenomena (42, 54). Lymphocytes play a predominant role in both types of response. Lymphocytes produce two types of daughter cells. Effector cells are short -lived cells which attack antigens,while memory cells are long-lived cells that retain the ability to start dividing on short notice to produce yet more effector and memory cells (54).

Effector cells from B-lymphocytes are responsible for Ig production. Immunoglobulins are produced in response to and react with an antigen (42, 54). They recognize and bind to foreign molecules, a process referred to as opsonization (42). Opsonization of microorganisms is considered the most important function of immunoglobulins in mammary secretions (69). Opsonization is the process by which Ig's, predominantly IgG, enhance ingestion of pathogens by phagocytes (42, 69). Other actions of immunoglobulins (IgA, IgM, IgG) include complement fixation and neutralization of toxins. Results of these actions include prevention of the adherence of bacteria to host tissue and agglutinization of pathogens (42).

Effector cells from T-lymphocytes do not secrete immunoglobulins, but instead possess specific surface molecules which recognize and react with antigens (42, 54). Effector T-cells also produce lymphokines. Lymphokines possess many biological characteristics such as chemotactic and mi-

gration inhibitory activity, and the ability to activate microbicidal mechanisms of phagocytic cells (42, 69).

Targowski (69) stated that, "The most important function of lymphocytes is to maintain the steady state (induction versus suppression) of the immune system through delicate cooperation among various populations of lymphocytes." The ability of lymphocytes to be sensitized through exposure to a specific antigen and maintain this sensitized state is significant to their role in the immune response. Bovine milk lymphocyte populations are composed of approximately 50% T-lymphocytes and 20% B-lymphocytes (69).

One line af attack to pursue in research to decrease mastitis incidence is to enhance the natural defense mechanisms discussed above. As mentioned previously the IMD and ICB have been used to enhance the phagocytic response by inducing leukocytosis (52). Nickerson (52) has discussed findings which indicate that commensal organisms such as *Corynebacterium bovis* which colonize the mammary gland may provide protection against major pathogens. These organisms appear to elevate SCC to a level sufficient to prevent infection by more pathogenic bacteria. Additionally, results from endotoxin induced leukocytosis studies (56, 70) have indicated similar benefits from elevated SCC. Methods of enhancement such as the IMD, ICB and endotoxin induced leukocytosis require repeated intervention from management throughout a cow's life. Methods of enhancement which do not require intervention such as increasing genetic resistance through selection would be preferable.

Genetic Resistance to Disease

Miller and Schultze (42) have defined genetic resistance as "some innate physiological defense mechanism of the animal which enables it to prevent pathogens from establishing an infection." They further stated that "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." Detection of the defense mechanism independent of infectious challenge and prediction of innate resistance prior to bacterial exposure is more desireable. However, lack of understanding of host defense mechanisms prevents early detection (42).

Selection for genetic resistance to mastitis is dependent upon two factors: 1) individual cow variation in the expression of the innate defense mechanism responsible for resistance and 2) the heritability of the measure of this defense mechanism (42). Genetic resistance to mastitis is most likely mediated by a large number of genes each having a small effect (42, 62). Heritability is the proportion of the total observed variation in resistance to mastitis which is accounted for by these genes. Without variation no progress can be made through selection.

In the previous discussion on defense mechanisms of the mammary gland, it was stated that resistant and susceptible cows differed in keritan composition (51). Additionally, cows have been reported to vary in phagocytic cell competence (42). Miller and Schultze (42) stated that the most likely source of this variation was genetic. Further work reviewed by Miller and Schultze (42) concerned selection for phagocytic ability of leukocytes in mice. Two-way selection for phagocytic ability of leukocytes yielded realized heritabilites of .30 for high ability and .25 for low ability. A correlated response to selection of higher spleen weights in the high response line was also observed. The lines did not differ in the ability of a single plasma cell to produce immunoglobulin, but the high line was found to be more susceptible to tumors (42).

Selection for resistance to Salmonella organisms in mice found that the line showing high resistance had high leukocyte counts while the line showing low resistance had low leukocyte counts (42). It was also found that mice resistant to Salmonella had an intracellular digestive enzyme in their macrophages that was absent in susceptible mice (42). Weir and Schlager (75) developed high and low leukocyte lines of mice through intense inbreeding. By generation 13 they had obtained a maximum, four fold difference between the lines. Average blood leukocyte counts in the high and low leukocyte lines were 17,500 and 4000 cells/ml. Heritability estimates for leukocyte count by

four methods were .22, .20, .19, and .17. Leukocyte numbers were found to confer resistance to irradiation but not to artificial infection with mouse typhoid (75).

In the F_2 generation of a cross between lines of mice differing in body size and leukocyte count, Chai (8) found that 50% of the variation in blood leukocyte counts could be attributed to a genetic effect. Subsequently, a directional selection experiment was started from hybrid stock. Selection was based on individual merit for high or low total leukocyte count. Following 11 generations of selection, Chai obtained lines that differed in both total (3 fold) and differential leukocyte counts. These lines also differed in body weight, reproductive performance and resistance to irradiation. Heritability estimates for leukocyte count ranged from .15 to .39 (8). Further work with these lines by Silver and Chai (64) revealed line differences in response to antigen. The low leukocyte line responded with predominantly IgM while the high leukocyte line produced almost exclusively IgG.

With regard to the genetic control of the immune response, Miller and Schultze (42) reported that virtually nothing is known of genetic control in the bovine. Commenting on two reports, they found: 1) various breeds and breed crosses of cattle did not differ significantly in blood concentrations of the various Ig classes and 2) genotypes for blood transferrin poymorphisms were found to be related to frequencies of positive CMT reactions (42). Much more is known of the genetic regulation of the immune response in laboratory species.

At least three sets of genes are involved in the control of humoral immunity in laboratory mice (42). One set of structural genes controls coding for the amino acid sequence of Ig molecules on B-lymphocytes. A second set of independent autosomal genes controls the quantitative function of antibody secreting plasma cells. Selection for high and low response to sheep red blood cells found a set of 10 separate loci to be involved in the quantitative response to antigen (42). The third set of genes regulates aspects of Ig function such as specificity and affinity. Selection for high and low affinity of antibodies to a specific antigen found these genes to be independent of those controlling antibody quantity(42).

At least two sets of genes have been found to be involed in the control of cell mediated immunity (42). The first set consists of structural genes which code for the composition of T-lymphocyte receptors. The second set consists of regulatory genes which govern antigen recognition functions of T-lymphocytes. These genes have been identified in many species and shown to be linked (on the same chromosome) with histocompatibility genes (42).

Further studies concerning genetic control of the immune response in mice have been aided by the discovery of the C3H/HeJ strain of mice. The C3H/HeJ strain is unique in its response to bacterial lipopolysaccharide (LPS) endotoxin. In contrast to other mice, C3H/HeJ mice possess a high degree of resistance to endotoxin lethality (73). Additionally, they do not support an *in vitro* mitogenic response to endotoxin and they produce only a small *in vivo* immune response to endotoxin (73).

Sultzer (67) studied leukocyte response in the peritoneal cavity after an intraperitoneal injection of endotoxin. He found leukocyte response to be a function of the relative toxicity of the endotoxin. Injection of an endotoxin dose toxic to susceptible mice resulted in a prolonged depression of mononuclear cells. Neutrophilia began after 12 hours, peaked at 24 hours and gradually receded over the next 48 hours. In C3H/HeJ mice this same dose produced only a mild depression of mononuclear cells for the first few hours followed by a substantial rebound which persisted for 72 hours. The neutrophil influx in C3H/HeJ mice was rapid, peaking at 12 hours and then subsiding. Increasing the endotoxin dose 100 fold, just below a lethal level in C3H/HeJ mice, produced a neutrophil response in C3H/HeJ resistant mice similar to that found in the susceptible mice with the lower endotoxin dose. Thus leukocyte response was shown to be a function of the relative toxicity of the endotoxin which, in turn, is dependent upon host susceptibility (67).

Moeller et al. (45) examined inflammatory response in relationship to endotoxin resistance in mice. In agreement with Sultzer (67) they observed a rapid neutrophil influx in C3H/HeJ mice which peaked at 12 hours. This was followed by an influx of macrophages which peaked at 48 hours. In susceptible mice maximum neutrophil and macrophage responses were not observed until 96

hours and were at least 80% less than those found in resistant mice. Other inflammatory agents failed to demonstrate differences between resistant and susceptible mice.

Moeller et al. (45) also found no differences between resistant and susceptible mice in serum complement titers, in the *in vitro* levels of chemotactic activity produced by LPS, or in the *in vitro* chemotactic capabilities of neutrophils. They found that the carbohydrate subunit of the LPS molecule was necessary for the preservation of the large differential inflammatory response seen in C3H/HeJ mice. As the polysaccharide content of endotoxin was successively decreased the inflammatory response generated in susceptible mice became more similar to that seen in C3H/HeJ mice.

Lastly, it was demonstrated that the survival of susceptible mice could be enhanced by increasing the magnitude of the intraperitoneal response with a simultaneous injection of a second inflammatory agent. Moeller et al. (45) concluded that the rapid influx of leukocytes in C3H/HeJ mice may be integrally related to their endotoxin resistance.

Verghese et al. (71) studied the genetic control of peripheral leukocyte response to endotoxin in mice. Changes in the number of circulating leukocytes at various times after LPS administration were measured as an *in vivo* manifestation of endotoxin susceptibility. Susceptible mice responded with a 50% drop in circulating leukocytes 3 hours after injection. Resistant mice of the C3H/HeJ strain required nearly a 100 fold dose to achieve a comparable change in circulating leukocyte numbers. F_1 mice from a susceptible × resistant cross were indistinguishable from the susceptible parent suggesting a dominant mode of inheritance for peripheral leukocyte response to endotoxin. This was confirmed through data from a backcross analysis (71).

Ruco et al. (55) reported the presence of a tumoricidal defect in macrophages of C3H/HeJ mice. Macrophages of C3H/HeJ mice failed to develop tumoricidal capacity after a variety of *in vivo* or *in vitro* stimuli. Further work by Ruco and coworkers (55) examined the control of macrophage tumoricidal capacity in relationship to the LPS gene. F_1 hybrids from a resistant × susceptible cross

produced a macrophage response intermediate to those of the parental strains. This suggested an autosomal codominant pattern of inheritance for the control of macrophage cytotoxicity (55). Backcross linkage analysis (C3H/HeJ × F_1) demonstrated complete concordance in the expression of macrophage tumoricidal capacity and spleen cell capacity to proliferate in response to LPS (55). Work with 8 strains of mice developed from the F_2 generation of a C3H/HeJ × susceptible cross revealed that the development of cytotoxic macrophages during infection was associated with the capacity of each strain to respond to LPS (55). The above findings of Ruco et al. (55) strongly suggest that gene control of macrophage tumoricidal capacity is either closely linked or identical to the LPS gene.

As mentioned previously, C3H/HeJ mice differ from their normal susceptible counterparts in that they do not support an *in vitro* mitogenic response to endotoxin and they produce only a small *in vivo* immune response to endotoxin (73). This discovery came from a study on the genetic control of response of mice to bacterial endotoxin (73). Backcross data from this same study revealed that the failure of C3H/HeJ mice to support *in vitro* mitogenic and *in vivo* immune responses to LPS appeared to be caused by a single gene effect. Further work by Watson and Riblet (74) revealed that the locus which restricts mitogenic and immune responses to LPS in C3H/HeJ mice also restricts polyclonal responses. In addition, results from this study revealed the following: 1) the failure of C3H/HeJ spleen cells to support responses to LPS is not due to nonspecific or LPS induced suppresive events, or to the lack of accessory cell types, 2) lymphocytes from C3H/HeJ and susceptible mice bind LPS equally well and 3) C3H/HeJ spleen cells respond normally to other B-lymphocyte mitogens (74). Watson and Riblet suggested that "C3H/HeJ mice have a defect in a membrane component that is activated via interaction with LPS and initiates the intracellular events that lead to cell proliferation."

From the preceding discussion of research on genetic resistance it is evident that much has been learned of the control of genetic resistance in mice. However, as was previously stated, little is known about the control of genetic resistance in the bovine. The direction which mastitis resistance research takes will be influenced by the results of work with the laboratory mouse. The selection

experiments of Weir and Schlager (75), and Chai (8) have demonstrated that peripheral leukocyte counts can be altered through selection. Given the role of leukocytes in the defense of the mammary gland, results such as these lead to the consideration of altering SCC through selection to increase resistance to mastitis.

SCC as a Measurement of Mastitis Resistance

From the previous discussion of the defense role of leukocytes during infection, it follows that milk SCC is a reflection of the health status of the mammary gland. The large influx of neutrophils which occurs during infection is reflected in the rise in SCC. In this way SCC is an indirect measurement of mastitis. Whether or not resistance to mastitis can be enhanced through selection to alter SCC depends upon the strength of the relationship between mastitis and SCC. The use of SCC as a selection criterion is in turn influenced by the ease and cost of measurement, and by the heritability of SCC.

SCC and Mastitis

The presence of neutrophils in large numbers in milk implies tissue injury. As mentioned before, during infection milk SCC is comprised of approximately 90% neutrophils (24, 51, 52). When the cause of infection is removed or irritant effects are reduced, neutrophil exudation correspondingly decreases. However, if tissue injury is extensive and complete healing requires weeks, even months, neutrophil exudation may continue long after the cause of infection is removed.

Given the relationship between mastitis and SCC during infection, Coffey et al. studied the association between initial SCC level in first lactation and the likelihood of developing mastitis later in the first and in subsequent lactations (10). Bacteriological data from this study suggested that cows with a low initial SCC in first lactation had a lesser risk of increased mastitis incidence later in first

and subsequent lactations than cows with a higher initial SCC. When examining differences between breeds in SCC, Grootenhuis et al. (22) found that Dutch Red and White cattle had higher cell counts and were more susceptible to mastitis than either Dutch or Holstein Friesian. Mastitis here was defined as a cell count greater than 500,000 cells/ml accompanied by a positive bacteriological test.

Lindstrom et al. (36) studied the predictive value of SCC by defining mastitis as a cell count greater that 250,000 cell/ml. For noninfected cows this definition of mastitis correctly identified 94 and 90 percent of the cows based on a single or combined quarter sample, respectively. This means that only 6 and 10 percent of the cows were incorrectly identified as infected. However, for cows that actually were infected, the proportions incorrectly identified were 26 and 50 percent for single or combined quarter samples, respectively. Therefore, the definition of mastitis based on SCC level identified healthy cows with fairly good accuracy but failed to recognize a considerable proportion of infected cows, especially when SCC was obtained from the combined quarter sample (36). This is important given that 70% of the cows had one quarter infected.

A genetic correlation measures the extent to which two characters are influenced by the same genes (19). Afifi (1) estimated the genetic correlation between mastitis and leukocyte count to be in the range of .83. In contrast, Coffey et al. (12) reported approximate genetic correlations between SCC and measures of infection to be in the range of .4 to .5. Based on their estimates, Coffey et al. commented that genes predisposing cows to low cell counts also result in lower rates of infection, but with frequent exceptions. Individuals with greater than average SCC and less than average rates of infection or the reverse would not be uncommon. Jones et al. (29) reported results similar to those found by (12). They found that the percentage of infected cows increased as SCC increased with greater infection rates occurring above 400,000 cells/ml. They also noted that neither bacterial cultures or SCC alone gave a true picture of an individual cow's status. Only 50 and 68 percent of the bacteriologically negative samples were below 100,000 and 200,000 cells/ml, respectively. Ten percent of the negative samples exceeded 800,000 cells/ml (29).

Measurement of SCC

Traits to be considered for selection should be measurable at low cost. It is also important that they be clearly defined and that observations be consistently recorded according to this definition (62). Milk SCC meets these criteria. In 1986 Shook (62) reported that more than 2.4 million cows, representing 50% of all Dairy Herd Improvement (DHI) cows were on somatic cell test. Dairy Herd Improvement associations routinely provide collection, measurement and recording of SCC for milk samples. The existing data base required for genetic evaluations is already extensive.

Heritability

Heritability is the ratio of additive genetic variance to total phenotypic variance. It expresses the extent to which phenotypes are determined by the genes transmitted from parents. Researchers have observed that considerable differences exist in the estimates of heritability found in the literature concerning resistance to mastitis (37, 59). Many of the differences result from the variety of definitions which are used for mastitis or mastitis resistance. The focus here will be on estimates of heritability for mastitis resistance based on SCC.

In 1952 Legates and Grinnells examined susceptibility to mastitis (35). A cow was labeled susceptible if at any time during a sampling period (1 to 6 years) she was found to have a leukocyte count of 500,000 cells/ml or greater accompanied by demonstrable streptococcus or staphylococcus organisms. A heritability estimate of $.27 \pm .10$ was obtained for susceptibility to mastitis (35).

More commonly, estimates for heritability are based on test day SCC or a lactation measurement obtained by combining several monthly SCC's. Monardes et al. (46) examined different methods of combining test day SCC for a single lactation and the heritability estimates obtained from these methods. Two scales were used for this evaluation: the unadjusted (1000 cells/ml) and SCC transformed to a natural logarithm scale. Heritability was higher for means transformed to the

logarithmic scale. Additionally heritability was slightly higher for lactation average SCC than for test day observations (.12 versus .08).

Manfredi et al. (37) reported heritability estimates for two lactation measurements of SCC: cumulative lactation score (CLS) and lactation somatic cell concentration (LSCC). With CLS infection was defined as a SCC over 400,000 cells/ml. The score was based on the number of monthly reports showing infection and their position along the lactation curve. The second measurement, LSCC, was the average of all monthly SCC measurements for the lactation. Heritability estimates for CLS and LSCC were .17 and .61, respectively. The authors could offer no explanation for the unusually high estimate for LSCC (37).

Shook et al. (63) reported a study performed to test the theory that the heritability of a lactation average SCC should be higher than the heritability of a single test. They found that heritability increased from .13 to .30 as the number of tests in the average increased from 1 to 4. Commenting on the increase in heritability, they stated that this was indicative of a relatively large variance among tests within a lactation and consequently a low repeatability of tests within a lactation (63).

Three additional estimates of heritability for lactation average SCC are .38 ± .20, .23 ± .22 (77) and .16 (61). As with the study by Shook et al. (63), these two studies utilized the natural log transformation. An extensive review by Miller and Schultze (42) in 1981 placed heritability for lactation average SCC at approximately .21. In 1986 Shook (62) reported that the heritability of lactation average SCC was more in the range of .10 to .15.

Several researchers have reported that the heritability of SCC increases with age (1, 11, 30). Kennedy et al. (30) examined the heritability of the natural log of test day SCC. Heritability increased from .05 for cows two years of age or less, to .10 for cows six years of age or greater. The average heritability for all ages was .08. Afifi (1) found lactation somatic cell concentration to be higher for fourth than first lactation cows (.37 versus .14).

Coffey et al. (11) examined heritability of lactation average SCC during first, second and third or later lactations. Lactation average SCC was transformed to a log base 2 scale and contained a minimum of 4 tests. Heritability estimates were $.09 \pm .02$, $.10 \pm .03$, $.29 \pm .03$ and $.18 \pm .02$ for first, second, third or later and mixed lactations, respectively. Given the increase of heritability with lactation age it was suggested that SCC early and late in life may be genetically different traits (11).

Relationship with Other Traits

The literature contains many reports of a negative relationship between SCC and milk yield (6, 12, 29, 30, 36, 39, 44, 70). Table 1 contains estimates of the genetic and phenotypic relationship between SCC and milk yield. From these estimates it is apparent that the general tendency for both genetic and phenotypic correlations is to be negative and near zero. Although the estimates are near zero, the observed decline in milk yield associated with increasing SCC is real and is cause for concern.

Jones et al. (29) studied the relationship between SCC and milk production relative to average herd production. They found the decline in milk yield with increasing SCC to be linear for herds averaging 7700 kg/yr. For herds with average production greater than 7700 kg/yr, the regression of test day on SCC was linear, quadratic and cubic. Commenting on their study, Miller and Paape (43) noted that the decline in milk yield with increasing SCC was rather startling even at low levels of SCC (50,000 to 100,000 cells/ml). Jones et al. (29) also reported that the impact of increasing SCC on milk yield was greater for second or later, than first lactation cows.

Meijering et al. (39) reported a study on the relationship between cell count and milk yield in which no clinical cases of mastitis were observed. They found that the losses due to subclinical mastitis as indicated by cell count can be substantial. Miller et al. (44) examined the relationship between SCC and yield relative to the bacteriological status of the cow. They found that bacteriological status of the cow did not have a significant impact on the decline in milk yield associated with in-

Table 1. Genetic and phenotypic correlations between SCC and milk yield.

Reference	Correlation	
	Genetic	Phenotypic
Manfredi et al. (47)	-0.11	0.02
	-0.01	-0.02
Kennedy et al. (30)	0.14	-0.13
Coffey et al. (11)	-0.28	-0.17
	-0.14	-0.12
Lindström et al. (36)		-0.08
		-0.02
		-0.11
		-0.07
Bodoh et al. (6)		-0.17

creasing SCC. In contrast, Afifi (1) reported that differences in cell counts among classes of average daily yield were not statistically significant in fourth lactation cows. However, he did note that very high and very low producing cows showed relatively higher cell counts than other groups.

Reports on the relationship between SCC and fat or protein are not as numerous in the literature. Table 2 contains estimates from three studies of the genetic and phenotypic correlations between SCC and fat or protein yield. As with milk yield, the relationship of SCC with fat and protein appears to be near zero. The genetic correlation with protein appears to be slightly positive while the pheotypic correlation appears slightly negative.

Variation in SCC and Factors Which Affect SCC

Along with the increase in SCC associated with infection, there are other factors which influence SCC. Syrstad and Røn (68) reported a study on variation in SCC. From an analysis of 2570 samples from 765 cows, proportions of the total variation were attributed to the following effects: Herds 13%, age groups 8%, cows (within herds and age groups) 31%, months 6% and residual 42%. The large individual cow variation implies that differences among cows are an important source of variation in SCC. The large residual variation represents differences among samples and the amount of changes in cell count occurring over time. Given that age and season effects are accounted for, the sample variation should also include stage of lactation effects.

In general, SCC tends to increase with age and stage of lactation, (4, 6, 42, 56) and to show no consistent relationship with season (24). A twelve year study by Blackburn (4) reported that as lactation age progressed from 1 to 7, average total cell count increased from 300,000 to 1,080,000 cells/ml. From the second lactation on, this increase was due primarily to an increase in neutrophils. Increased SCC's with age have been attributed to the accumulative effects of bacterial infection and stress of milking (56). However, Blackburn (4) noted that while approximately 5% of all quarters were infected for most of the 12 years, for about 12 months of the study cows were

Table 2. Genetic and phenotypic correlations between SCC and fat or protein.

Reference	Fat Yield		Protein Yield	
	rg	rp	rg	rp
Manfredi et al. (37)	-0.02	0.07	0.08	-0.05
Kennedy et al. (30)	0.08	-0.13	0.18	-0.11
Coffey et al. (12)	-0.09	-0.10		

free of infection and during that time the average total cell count was approximately equal to that of first lactation animals (300,000 cells/ml). The average lactation age during that time was 4.3 lactations.

With regard to stage of lactation, Bodoh et al. (6) observed no differences in SCC for the first three stages of lactation, but noted a sharp rise in the fourth and again in the fifth stage of lactation. Stage of lactation was one of four 61 day periods postpartum or a fifth period more than 244 days postpartum. Cell counts did not rise as sharply in younger animals relative to the rise in older animals. Jones et al. (29) found the first test during lactation to be high, followed by a decrease in SCC and then an increase throughout lactation from the third test onward. High counts early in lactation have been attributed to new infections while those in late lactation have been attributed to chronic and subclinical infections (42). Additionally, the drop in milk volume with advancing lactation is thought to concentrate cells in a smaller volume of fluid resulting in higher SCC (56, 63).

Two further sources of variation in SCC are species of pathogen and management practices. Ward and Schultze (72) reported differences in cell counts associated with infections caused by different types of bacteria. They also found that cows which had exhibited prior clinical mastitis at some point in their life had higher counts, even with no current infection. Management practices influence SCC indirectly through their impact on new infections and infection rates. Bodoh et al. (6) reported that herds with milking parlors had lower counts than those with barn pipeline systems. They also reported that herds using teat dip and selective dry cow therapy exhibited the lowest counts.

The Use of Laboratory Mice in Dairy Research

The use of cattle for genetic and mastitis research is hindered by problems of husbandry which include management, cost, and generation interval. Use of the laboratory mouse has been proposed for the study of genetic and physiological effects of selection for increased milk production. The laboatory mouse has also been proposed as a model for bovine mastitis research. Characteristics of the mouse which make it a desirable model for dairy research are it's low cost of maintenance, prolificacy, and short generation interval (3 months).

Enzman in 1933 (17) reported a milk production curve for laboratory mice. The general shape was quadratic, symmetrical between days 5 and 12 of lactation and peaking between 8 and 10 days postpartum. Hanrahan and Eisen (23) in 1970 obtained a lactation curve by direct milking procedures. They also reported a quadratic shape; A second degree polynomial accounted for 41% of the observed variation. However, their curve peaked slightly later between 12 and 13 days.

Cole (13) and Munford (47) both studied changes in the mammary gland of the mouse during lactation. Cole in 1933 observed that the amount of glandular tissue appeared to reach a maximum about 12 days postpartum (13). Similarly, Munford in 1963 reported that tissue activity in the mammary gland reached a maximum between 10 and 13 days postpartum (47). From the above studies it can be seen that peak production corresponds with the peak in tissue activity of the gland.

Based on the results of his study in 1947 Falconer (18) proposed litter weight at 12 days as a more convenient method of measuring milk production than direct milkng procedures. Using growth during suckling as a measurement of milk production had the advantages of allowing lactation to proceed naturally and it was simple to carry out. It's disadvantages were that it was an indirect measurement and only part of the milk produced was recorded for not all milk consumed by the young is used for growth (18).

Bateman (2) later examined the suitability of Falconer's indirect measure of milk production. The validity of Falconer's procedure rested on the supposition that early growth of young mice is determined largely by the mother and only slightly (if at all) by the intrinsic growth rates of the young (2). Bateman found that 73% of the total variation in 12 day litter weight could be attributed to maternal influences. However, only 32% was attributable to postnatal maternal effects.

Cox et al. (14) reported the results of a cross foster study which found maternal influences to be the most important single factor in determining body weight through weaning. For days 5, 12, and 21 postnatal maternal influences accounted for 53, 62 and 60 percent of the observed variation in individual body weight. In contrast to Bateman (2), Cox et al. found that 71.5% of the variance in 12 day litter weight was accounted for by postnatal maternal effects. Young et al. (78) also performed a cross foster study. They too found that postnatal maternal influences accounted for a major proportion of the variation in litter weight at 12 days (approximately 80%).

Jara-Almonte and White (27) used daily milk records to characterize the shape of the lactation curve in mice and to estimate lactation milk yield. Milk yield for days 6 through 21 was estimated by isolating litters from their dams for 6 hours and then weighing the litters before and after 1.5 hours of suckling. Regression of average daily milk yield on day of lactation produced second and third degree polynomials which accounted for 92.3 and 98.6 percent of the observed variation in milk, respectively.

Jara-Almonte and White (27) reported peak production on days 13 and 14 for the second and third degree polynomials. This compared favorably with the results of Hanrahan and Eisen (23), but maximal yield per dam per day of 3.1 grams was considerably higher than the maximal yield of approximately 1.8 grams reported by Hanrahan and Eisen. Measurement of milk yield on days 8, 10, 13, 15, 18 and 20 was reported to provide an excellent prediction (R^2 = .86) of actual yield (27).

Chandler (9) in 1970 investigated the suitability of the laboratory mouse as a system for studying bovine mastitis. He produced experimental mastitis through intramammary innoculation of

organisms commonly associated with bovine mastitis. The microscopic and histological responses observed closely resembled those that occur in the cow. There was evidence that the spectrum of pathogenicity of different bacterial strains in the bovine species was to some extent parallelled by the difference in pathogenicity in the mouse. Additionally, results from dye innoculations and 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.

Kokkalis (33) reported the distribution of SCC in the milk of laboratory mice. Cell count was measured on days 9, 14 and 18 of a 21 day lactation. A large stage of lactation effect was reported, SCC on day 14 being slightly higher and SCC on day 18 much higher than those on day 9 of lactation. Table 3 contains the distribution of SCC in dairy cattle and mice. Utilizing data from days 9, 14 and 18 SCC in the mouse is approximately 5 times that found in the cow. Removal of day 18 counts which were typically much greater than earlier counts brings the distribution in mice down to approximately 4 times those found in dairy cattle (32).

Table 3. Distribution of somatic cell counts (SCC) in milk of cows and mice[4].

Cows		Mice			
		Days 9, 14, 18[1]		Days 9, 14[2]	
SCC[3]	(%)	SCC[3]	(%)	SCC[3]	(%)
≤ 100	36	≤ 500	35	≤ 400	35
100-200	21	500-1000	18	400-800	24
200-300	12	1000-1500	10	800-1200	12
300-400	8	1500-2000	6	1200-1600	7
400-500	5	2000-2500	4	1600-2000	5
> 500	18	> 2500	27	> 2000	17

[1]n = 2727

[2]N = 1818

[3]cells/ml

[4]Adapted from Kokkalis (32).

Materials and Methods

In June of 1984 a bidirectional selection experiment was initiated in the genetics mouse laboratory of the Department of Dairy Science at Virginia Tech. Three reproductively distinct lines were randomly assigned from existing laboratory stock. Fifty litters were assigned to a line to be selected for low milk SCC (LSCC), fifty to a line to be selected for high milk SCC (HSCC) and thirty-six to a control line (CSCC). Procedures for the first seven generations of selection have been described by Kokkalis (32). Procedures for the second half of the experiment have remained the same and are described below.

Management

Mice were maintained in opaque polypropelene cages approximately 27 cm × 17 cm × 13 cm. Metal cage tops served as feed hoppers and held water bottles. Bedding consisted of a natural cellulose material derived from corn cobs[1]. Bedding was changed weekly. A high energy feed[2] was fed from the time of mating until pups were weaned. This feed contained 17% crude protein, 11%

[1] Andersons Cob Division, Maumee, Ohio.

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

crude fat, 3% crude fiber, 6.5% ash, 2.5% minerals and a full compliment of vitamins. From weaning until next mating pups were fed a lower energy feed[3] which contained 22% protein, 5% fat, 5% fiber, 6% ash and a full compliment of minerals and vitamins. The temperature in the laboratory was maintained at approximately 22° C with 50 to 60% relative humidity and a 1:1 light dark ratio.

Laboratory Procedures

Matings and Litter Standardization

Selected lines (LSCC and HSCC) were perpetuated by 50 single pair matings and the control line (CSCC) by 36 single pair matings each generation. Replacement matings were available for each line to be used in the event of the death of one or both mice in a pair.

Matings within each line were at random with the exception that full sib matings were avoided. Only virgin males and females 6 to 9 weeks of age were used. Matings were made in three groups to distribute the work load over time. Mating groups were one week apart and contained one third of the matings from each line.

Males were removed 10 days after mating and discarded. Eighteen days after mating females were checked daily for littering. At littering the following information was recorded: line number, litter number, sire and dam number, birth date, mating group and number born alive. Generation number was denoted by the first 2 digits of the 5 digit sire and dam numbers.

At 8 days of age, where possible, litters were standardized to 5 males and 5 females by removing excess pups or augmenting with foster pups. Foster pups were excess pups from the same line born

[3] Agway prolab rat/mouse/hamster 3000, from Agway Inc., Country Foods Division, Syracuse, New York.

on the same day. They were tail clipped for ease of identification and later discarded at weaning. Litters containing less than 6 pups and those which could not be augmented to at least 8 pups were recorded and discarded. The purpose of standardization was to stimulate maximum milk flow in the dam (3).

At 12 days of age non-fostered mice were individually identified by toe notching and sex was confirmed. This information was recorded along with litter size at day 12. Litters were weaned at day 21 and dams discarded.

Measurements

SCC: All lactating females were milked on days 9, 14 and 18 of their 21 day lactation. To increase sample yield, litters were removed from their dams 3 to 5 hours prior to milking. Immediately preceding milking, females were anesthetized with methoxyflurane and injected with 1 USP oxytocin. Milk was obtained with a vacuum-operated milking device (Figure 1) developed from that described by McBurney et al. (38). A capillary tube extending from the device was placed over the teat end and vacuum (100 mm Hg) applied by covering the air hole on the side of the device. Several teats were sampled to obtain at least 30 μl of milk.

A microliter pipette was used to transfer 25 μl of milk to a 1.5 ml microfuge tube containing 225 μl of physiological saline. Samples were mixed with an electrical mixer and SCC counted by a Fossomatic[4] cell counter. A saline control sample was counted with milk samples collected each day to adjust for machine variation.

Any negative cell counts resulting from correction for machine variation, as well as cell counts of zero, were converted to one to permit logarithmic transformation. Cell counts were multiplied by 10 to adjust for dilution and by 1000 to express counts in cells/ml of milk. Collection, dilution,

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[4] A/S N. Foss Electric, Hillerod, Denmark.

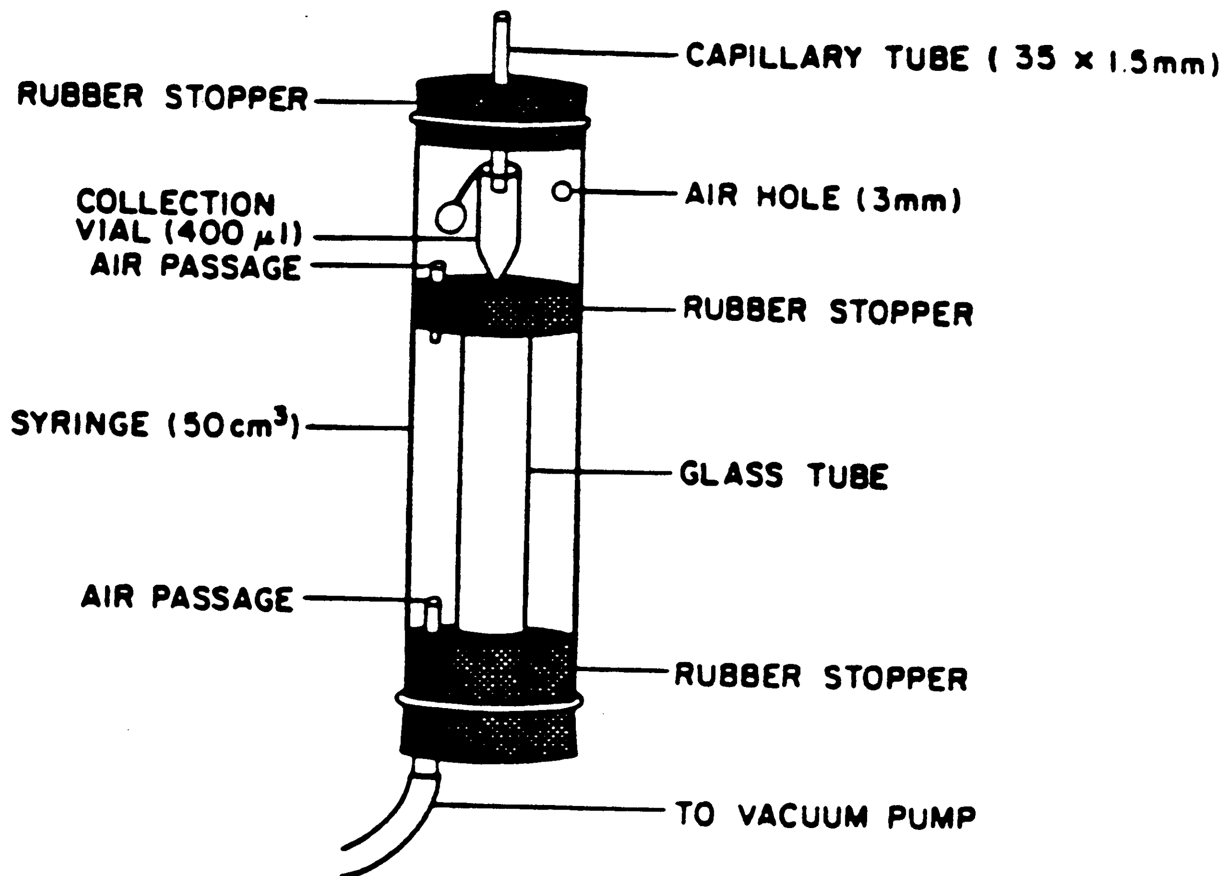

Figure 1. Diagrammatic representation of device used to obtain milk samples for somatic cell count determination.

mixing and counting procedures were reported to yield a repeatability of .99 (n = 49) for split samples of the same mouse-day (32).

A lactation average SCC was calculated from the individual days (9,14, and 18) after transformation to log base 2 and adjustment for stage of lactation (adjustment to day 14 basis). Adjustment was made because of a large stage of lactation effect discovered early in the experiment (32).

Milk Yield: Yield measurements were taken on days 8, 13, 17 and 20 of lactation. The weigh-suckle-weigh procedure described by Jara-Almonte and White (27) was used to determine milk yield. Litters were separated from their dams for a period of 6 hours during which they had no access to food or water. Following separation litters were weighed, returned to their dams to suckle for 1.5 hours and then weighed once more. Milk yield for that day was calculated to be the difference in litter weight before and after suckling. No adjustment was made for losses in urine and feces during the suckling period. Following 6 hours of fasting the alimantary tract of the pups was expected to be nearly empty (3).

Differences in litter weight which were less than zero were set equal to zero. Observations with yields of zero on day 8, 13 or 17 were discarded before analyzing milk yield data. Observations with yields greater than 3 standard deviations above or below the mean for that day were also discarded prior to analysis. Approximately 30% of the total number of records from 14 generations were discarded as a result of these two data edits.

Depending upon the available data, total lactation milk production was estimated for each female from yields at days 8, 13, 17 and 20, or days 8, 13 and 17 using regression equations described by Jara-Almonte (26). The accuracy (R^2) of total milk yield estimation from the equations using 4 and 3 days were .76 and .67, respectively (26). Beginning in generation ten, yield measurements were reduced by selecting a random sample half of the available litters.

E. Coli Endotoxin Trials: *E. coli* endotoxin trials began in generation three. Their purpose was to study line differences in the ability to mobilize phagocytic cells. In generation three 18 females were randomly chosen from each line to be challenged on day 11 or 16 of lactation (9 per day). In generation four and thereafter, the number of challenge mice was increased to 24 per line (12 per day).

Challenge mice were anesthetized and 3 glands (R4, R5 and L1) were washed with 70% alcohol and each injected with .05 ml of endotoxin in physiological saline (.1 mg/ml). Twenty-four hours after injection, mice were milked and SCC determined as previously described. Challenge response was calculated to be the difference between SCC on the challenge and previous non-challenge day (day 9 for challenge day 11; day 14 for challenge day 16).

Differences in SCC less than zero or equal to zero were set equal to one to permit logarithmic transformation. The appropriate concentration of endotoxin and post injection time of milking were determined through preliminary experimentation (32).

Blood Samples: Blood samples began in generation two. Eighteen females were randomly chosen from each line to be sampled on day 7, 15 or 21 (6 per day). In generation four and thereafter the number of mice sampled was increased to 24 (8 per day). Females chosen for blood sampling were the same mice assigned for endotoxin challenge. Mice to be sampled were anesthetized and .02 ml of blood collected via the tail vein. Blood was transferred by capillary tube to 1.98 ml of glacial acetic acid solution anticoagulant. Total white blood cell counts were determined by haemocytometer. Slides for differential counts (neutrophils, lymphocytes, monocytes, and eosinophils) were also prepared. Blood samples were processed by the Virginia-Maryland Regional College of Veterinary Medicine.

Selection

In the selected lines (LSCC, HSCC), entire litters were selected based on the average breeding value of their parents. The measure of performance used in calculating breeding values was the average SCC (log base 2 transformed and adjusted for lactation stage) of 1 to 3 measurements deviated from the corresponding line, generation mean:

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

where:

- $X_{ijk} =$ the measure of performance of the kth female in the jth line and i^{th} generation.

- $\overline{Y}_{ijk} =$ the mean SCC of the kth female in the jth line and i^{th} generatation.

- $\overline{Y}_{ij} =$ the mean SCC of all females in the jth line and i^{th} generation.

Transmitting abilities for sires were computed from a 6-member selection index 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 performance of the sire's dam.

- $X_2 =$ the mean performance of the sire's full sisters.

- $X_3 =$ the performance of the sire's maternal granddam.

- $X_4 =$ the performance of the sire's paternal granddam.

- $X_5 =$ the mean performance of the full sisters of the sire's sire.

• X_6 = the mean performance of the full sisters of the sire's dam.

• b's = selection index weights based on the amount and source of information (assumed heritability for SCC of .10).

Transmitting abilities for dams were calculated using identical relatives of the dam together with her own measure of performance. Three generations were required to obtain a full complement of relatives. Therefore, parents of the first three generations were selected on partial indexes from measurements of available relatives (32).

Litters from parents having the lowest average breeding values were chosen to be parents in the LSCC line, while litters from parents having the highest average breeding values were chosen to be parents in the HSCC line. With a full complement of relatives and an assumed heritability for SCC of .10, the theoretical accuracies of these indices were approximately .4 and .5 for sires and dams, respectively (32).

To minimize inbreeding effects, a maximum of 4 males and 4 females were used as parents from any single litter. This restriction together with an average standardized litter size of approximately 10 mice yields a selection intensity of approximately 25%. Based on a selection intensity of 25%, a theoretical change of approximately .17 standard deviations in average log base 2 SCC per generation was expected (32).

Parents in the control line were selected at random, without regard to family structure. Matings in all lines were at random except for the avoidance of full sib matings. After the selection of parents for the next generation, all remaining mice were discarded.

Statistical Procedures

Direct Response to Selection for Milk SCC

Line generation means for lactation average $\log_2$ SCC were computed for all lines (LSCC, HSCC and CSCC) and regressed on generation number (13 generations). Regression coefficients represented the average change in SCC per generation. Additionally, line generation means for lactation average $\log_2$ SCC of the LSCC line were deviated from those of the HSCC line and regressed on generation number. This regression represented the average divergence between the selected lines per generation. All regression coefficients were tested for significance by t-tests.

The regression of line generation mean performance on the cumulative genetic selection differential was also computed. This regression is similar to a realized heritability except that an assumed heritability of .10 was accounted for in the determination of the selection index estimates of transmitting ability. Thus selection differentials were genetic rather then phenotypic and the expected value of the regression of performance on cumulative genetic selection differential is actually 1.0 for true heritability of .10. Line generation means for lactation average $\log_2$ SCC of line LSCC were deviated from those of line HSCC. Genetic selection differentials for lines LSCC and HSCC were computed by deviating the mean line generation breeding value of selected parents from the appropriate mean line generation breeding value of all individuals in that line generation. The deviated cumulative genetic selection differential (HSCC - LSCC) was then computed over 13 generations. The deviated line generation means (HSCC - LSCC) for SCC were then regressed on the deviated cumulative genetic selection differential.

Correlated Response to Selection

Average response in each of the seven secondary traits (milk yield, total white blood cell count, the percentage of phagocytic cells in the blood, response to *E. coli* endotoxin challenge, the percentage of females mated that actually littered, the number of young born alive per litter and the percentage of young per litter which survived to weaning) was determined by computing line generation means for each trait in all lines and regressing the means on generation number. Deviations of the form HSCC - LSCC were also computed and regressed on generation number. Regression coefficients were tested for significance by t-tests.

Additionally, in the same manner as was described in the previous section, line generation means for line LSCC were deviated from those of line HSCC and regressed on the deviated cumulative genetic selection differential.

Phenotypic Correlations

Phenotypic correlations were computed among the following traits: SCC, milk yield, white blood cell count, percentage of phagocytic cells in the blood, response to *E. coli* endotoxin challenge, the number of young born alive per litter and the percentage of young surviving to weaning. Correlations were computed after adjusting for the following effects: line, generation, mating group within generation and line by generation interaction. Correlation coefficients were tested for significance by t-tests.

Results and Discussion

Direct Response to Selection for SCC

The principle question addressed by any selection experiment concerns the magnitude of response in the criterion of selection. Table 4 contains line generation means for lactation average $\log_2$ SCC of the selected and control lines over 14 generations of selection. The same information is displayed graphically in Figure 2. Given that divergent selection was practiced one would expect to find an upward trend for the HSCC line and a downward trend for the LSCC line. The response in the control line (CSCC) would be expected to fall between the responses of the 2 selected lines. However, in Figure 2 it can be seen that the generation means moved erratically in all lines with little separation between selected lines. In general, the response of the HSCC line was slightly greater than that of the LSCC line. The response in the control line was very similar to that of the HSCC line.

The simplest measure of average, per generation response to selection is the regression of performance on generation number. As would be expected from the results depicted in Figure 2, the average response in SCC per generation was quite small in both selected lines (.0678 $\pm$ .0341 for the HSCC line and .0384 $\pm$ .0390 for the LSCC line) and not significantly different from zero ($P >$

Table 4. Line generation means for somatic cell count (SCC) of selected (LSCC, HSCC) and control lines (CSCC).

	LSCC			CSCC			HSCC		
	Number of	Log_2 SCC		Number of	Log_2 SCC		Number of	Log_2 SCC	
Generation	Mice	Mean[1]	SD[2]	Mice	Mean[1]	SD[2]	Mice	Mean[1]	SD[2]
1	35	19.49	1.13	28	20.10	1.18	35	19.77	0.98
2	47	18.38	1.04	33	18.28	1.07	50	18.61	1.37
3	44	18.60	1.09	31	19.06	1.18	44	19.13	1.24
4	43	19.26	1.60	33	19.30	1.05	45	19.12	1.58
5	41	19.54	1.45	32	19.82	1.01	45	19.96	1.65
6	41	18.47	1.68	29	18.81	2.07	46	18.88	1.98
7	48	19.55	1.94	28	20.03	1.15	40	20.09	2.09
8	37	19.16	0.95	32	19.87	1.07	29	20.09	0.88
9	38	18.11	1.12	27	18.67	1.25	35	18.91	1.40
10	45	18.59	1.17	35	19.24	1.13	41	19.36	1.09
11	36	19.60	0.87	40	19.75	0.91	46	19.93	1.10
12	25	19.75	1.08	35	20.53	1.24	34	20.36	1.18
13	35	18.81	0.93	24	19.03	0.94	47	19.59	0.98
14	34	19.97	0.87	33	20.36	0.82	40	20.25	0.62

[1]Mean SCC measured in cells/ml and expressed on a log_2 scale.

[2]Standard deviation of the mean.

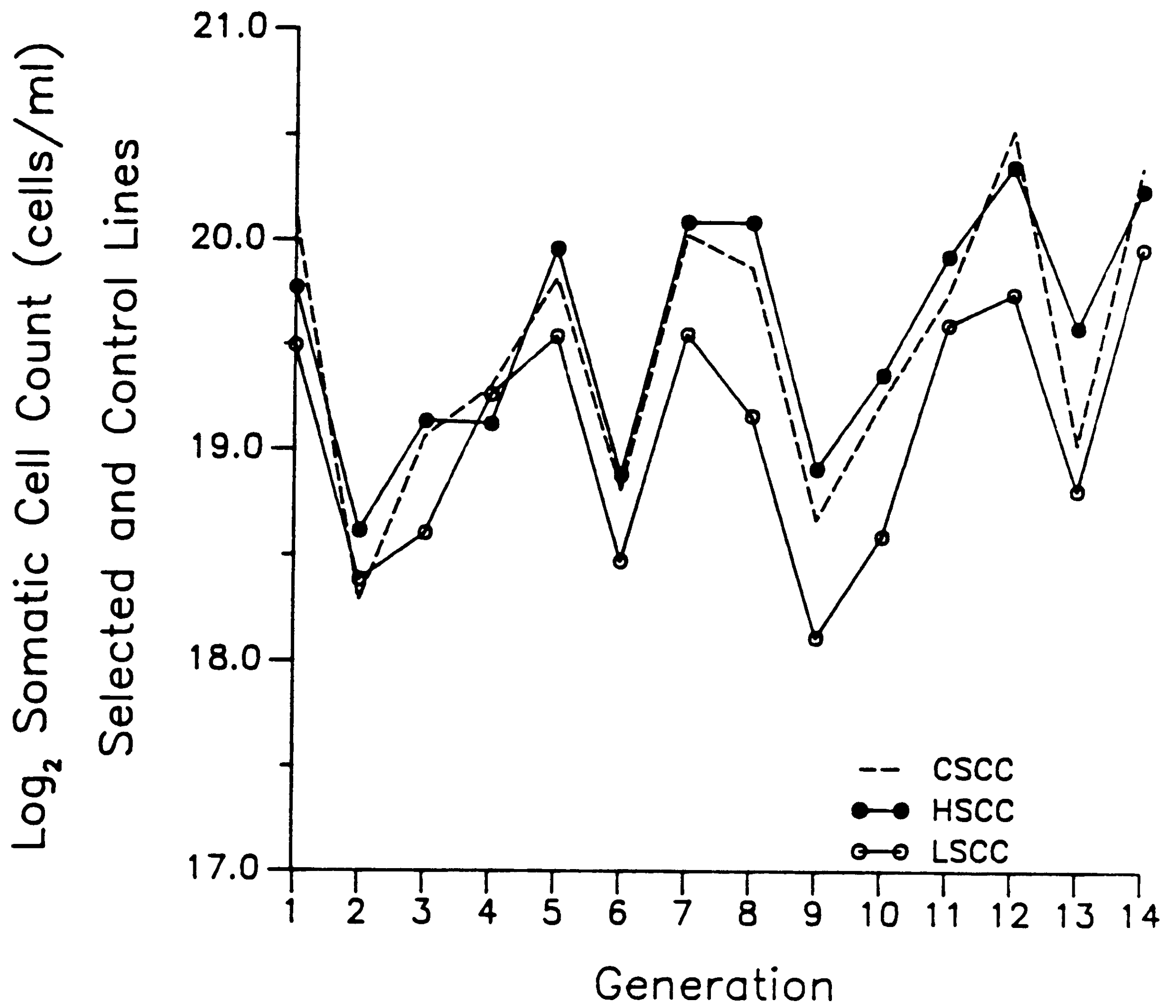

Figure 2. Response to selection for $\log_2$ SCC.

.05). The average change per generation in the control line was similar to that of the HSCC line (.0539 ± .0439) and also not significantly different from zero (P > .05). Another estimate of direct response is the regression of the deviation between two lines which have undergone divergent selection on generation. This regression yielded an estimate of the average divergence per generation which was quite small (.0294 ± .0178) and not significantly different from zero (P > .05).

Poor response to selection led to examination of the estimated breeding values for all and for selected individuals. Table 5 contains line generation means for estimated breeding values of all individuals in a given line generation, those individuals whose offspring were selected to be parents in the next generation, and the genetic selection differentials. Mean breeding values for the first three generations were omitted because they were calculated from partial indexes. From the genetic selection differentials for lines LSCC and HSCC it is apparent that selection procedures did succeed in choosing the more extreme individuals in these lines. However, genetic selection differentials for the control line indicate that the more extreme individuals for high SCC were also chosen to continue the control line. Investigation of selection procedures for the control line revealed that an error was made which caused the controls to receive positive selection pressure for SCC.

Table 6 contains the phenotypic performance for the corresponding mean line generation breeding values listed in Table 5. From the phenotypic selection differentials for lines LSCC and HSCC it is again apparent that selection procedures succeeded in choosing the more extreme individuals, with the exception of generation 11 for line LSCC. In all 3 lines the phenotypic selection differential for generation 11 were relatively near zero. This result reflects an error in selection which occurred in generations 10 and 11 and caused the number of dams available for evaluation to be effectively halved. Therefore SCC in line LSCC following generation 10 is most likely higher than would otherwise have been had the error in selection not occurred. This might explain the slight upward trend in SCC for line LSCC displayed in Figure 2. Also as a result of the error in selection SCC in line HSCC and in the control should be slightly depressed following generation 10.

Table 5. Estimated breeding values and genetic selection differentials for $\log_2$ SCC in selected (LSCC, HSCC) and control lines.

Generation	LSCC			HSCC			CSCC		
	All	Selected	Diff.[1]	All	Selected	Diff.[1]	All	Selected	Diff.[1]
4	-0.089	-0.175	-0.086	0.102	0.225	0.123	0.049	0.114	0.065
5	-0.103	-0.165	-0.062	0.078	0.197	0.120	0.076	0.122	0.046
6	-0.090	-0.212	-0.123	0.127	0.260	0.133	0.084	0.189	0.105
7	-0.152	-0.250	-0.098	0.156	0.272	0.116	0.130	0.175	0.045
8	-0.121	-0.179	-0.059	0.159	0.196	0.037	0.076	0.139	0.063
9	-0.132	-0.204	-0.072	0.078	0.157	0.080	0.066	0.110	0.044
10	-0.077	-0.120	-0.043	0.093	0.112	0.019	0.055	0.080	0.025
11	-0.075	-0.095	-0.020	0.047	0.095	0.048	0.038	0.057	0.020
12	-0.023	-0.070	-0.048	0.014	0.075	0.061	0.031	0.103	0.073
13	-0.023	-0.065	-0.043	0.035	0.098	0.064	0.066	0.106	0.040

[1]Genetic selection differential (Selected-All).

Table 6. Line generation means for $\log_2$ SCC of selected and unselected individuals.

Generation	LSCC			HSCC			CSCC		
	All	Selected	Diff.[1]	All	Selected	Diff.[1]	All	Selected	Diff.[1]
4	19.26	18.30	-0.96	19.12	20.36	1.24	19.30	19.95	0.65
5	19.54	18.31	-1.23	19.96	21.03	1.07	19.82	20.35	0.53
6	18.47	17.30	-1.17	18.88	20.15	1.27	18.81	20.06	1.25
7	19.55	18.35	-1.20	20.09	21.07	0.98	20.03	20.68	0.65
8	19.16	18.56	-0.60	20.09	20.53	0.44	19.87	20.58	0.71
9	18.11	17.35	-0.76	18.91	19.67	0.76	18.67	19.39	0.72
10	18.59	18.64	0.05	19.36	19.65	0.29	19.24	19.37	0.13
11	19.60	19.41	-0.19	19.93	20.21	0.28	19.75	20.19	0.44
12	19.75	19.41	-0.34	20.36	20.95	0.59	20.53	21.35	0.82
13	18.81	18.33	-0.48	19.59	20.37	0.78	19.03	19.30	0.27

[1]Phenotypic selection differential (Selected - All).

As with genetic performance (Table 5), phenotypic performance (Table 6) also indicates that the control line underwent postive selection for SCC. Phenotypic performance for SCC in the control line was greater than line LSCC and relatively similar to performance in line HSCC.

Correlated Responses to Selection for SCC

Milk Yield

In dairy cattle, both the genetic and phenotypic relationships between milk SCC and milk yield are negative (see Table 1). If a similar negative relationship exists in mice, it would be expected that alteration of SCC through selection would be accompanied by a corresponding change in milk yield. Line generation means for lactation milk production of selected and control lines are presented in Table 7 and the same information is graphically displayed in Figure 3. From Figure 3 it is evident that all lines experience a decline in milk yield across generations. The average response in the selected lines was -.2306 ± .0991 gms for the HSCC line and -.3100 ± .0792 gms for the LSCC line. The average change per generation in the control line was -.2631 ± .0938 gms. All regression coefficients were significantly different from zero ($P < .05$). However, there was no trend toward separation in the two selected lines. The average divergence between the 2 selected lines was .0794 ± .0789 and not significantly different from zero ($P > .05$). Given the vritual lack of response to selection for SCC, it is not surprising that there should be no divergence between the selected lines for milk yield. The decline that is evident in all 3 lines may result from a deterioration in the environment or possibly from a deterioration in maternal fitness as measured by milk yield.

Table 7. Line generation means for lactation milk yield of selected (LSCC, HSCC) and control (CSCC) lines.

Generation	LSCC			CSCC			HSCC		
	Number of Mice	Mean[1]	SD[2]	Number of Mice	Mean[1]	SD[2]	Number of Mice	Mean[1]	SD[2]
1	32	26.74	5.50	23	25.47	4.25	28	25.89	4.58
2	40	29.68	5.47	26	28.41	5.15	39	30.11	11.57
3	36	25.96	5.63	28	25.46	5.34	41	25.74	5.25
4	40	28.42	6.27	31	25.53	4.30	36	25.84	5.52
5	36	28.91	5.28	23	28.16	5.32	33	28.75	7.04
6	35	26.62	5.90	23	23.64	5.38	36	27.47	6.27
7	39	26.74	6.23	24	24.90	5.38	36	24.96	4.57
8	33	27.61	5.06	24	26.72	6.22	25	25.87	7.15
9	33	25.51	4.39	16	24.55	3.80	24	24.62	8.85
10	18	25.27	5.05	18	23.59	6.18	17	23.80	3.85
11	16	24.44	4.93	15	25.38	5.66	23	25.81	5.07
12	14	26.09	4.62	15	24.57	5.22	17	26.25	4.75
13	15	24.07	6.44	11	24.33	6.43	18	25.27	5.09
14	17	24.28	4.16	16	21.82	4.13	19	24.07	4.46

[1]Milk yield in grams.

[2]Standard deviation of the mean.

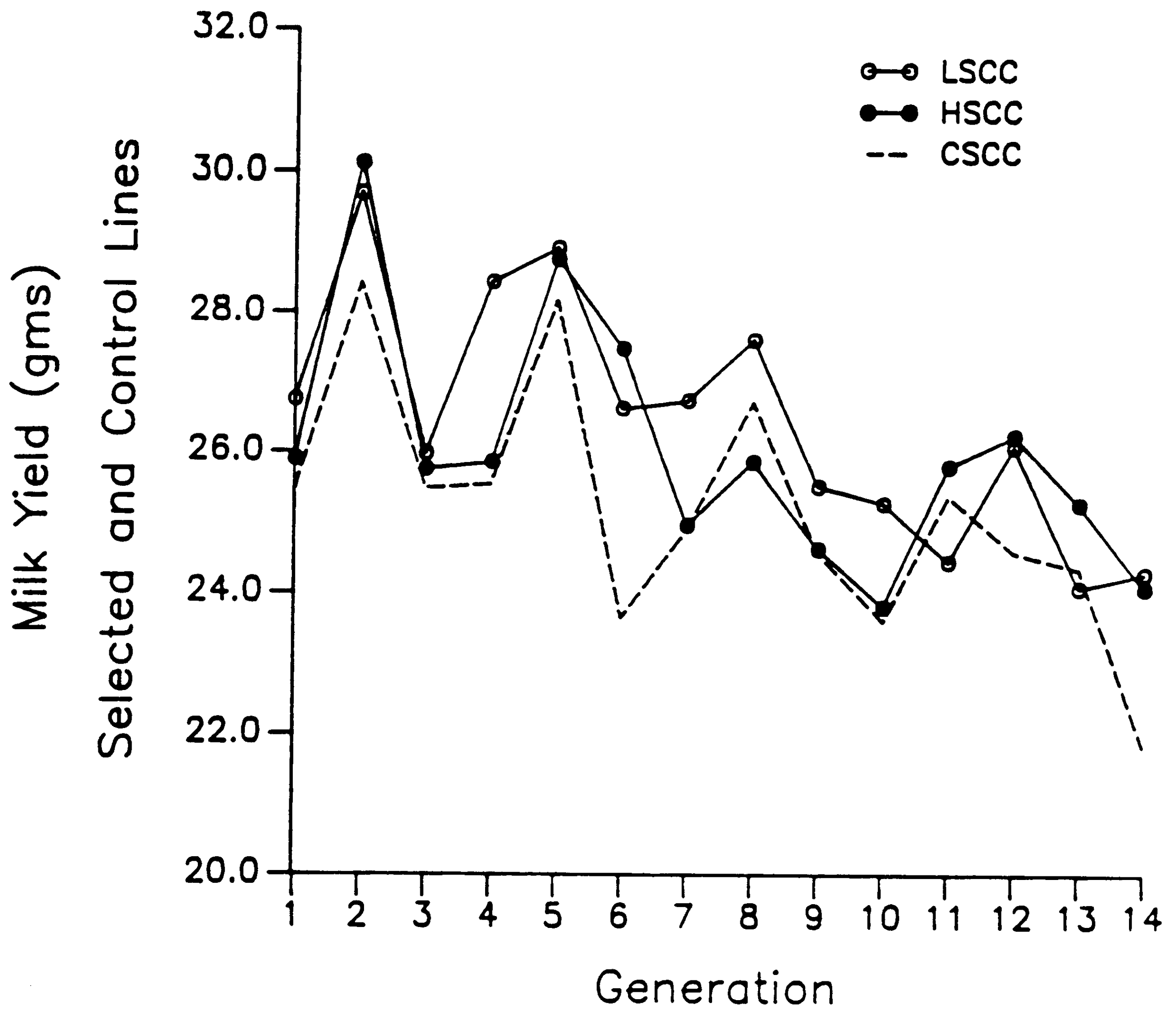

Figure 3. Change in lactation milk yield (gms) over time.

Total White Blood Cell Count

Milk SCC's are mainly composed of leukocytes derived from the blood. Blood leukocyte levels were measured to determine if alteration of milk leukocyte levels are a reflection of changes occurring in the blood leukocyte pool. Table 8 contains the line generation means for $\log_2$ total white blood cell count in the selected and control lines. Figure 4 contains a graphic display of the same information. There was little fluctation in total white blood cell count for all 3 lines. Average response per generation in the selected lines was .0093 ± .0159 for the HSCC line and .0033 ± .0092 for the LSCC line. The average change per generation in the control line was -.0134 ± .0111. No regression coefficients were were significantly different from zero ($P > .05$). Given the virtual lack of selection response in SCC it is not surprising that there are no changes expressed in the blood leukocyte levels. Additionally, there was no significant divergence between the selected lines (.0060 ±; $P > .05$). However, white blood cell counts in the HSCC line and the control were generally greater than those in the LSCC line.

Table 8. Line generation means for $\log_2$ total white blood cell count of selected (LSCC, HSCC) and control lines (CSCC).

	LSCC			CSCC			HSCC		
	Number of	$\log_2$ SCC		Number of	$\log_2$ SCC		Number of	$\log_2$ SCC	
Generation	mice	Mean[1]	SD[2]	mice	Mean[1]	SD[2]	mice	Mean[1]	SD[2]
3	16	22.80	0.68	16	23.17	0.61	15	23.38	0.52
4	17	22.91	0.45	18	23.19	0.72	17	23.06	0.40
5	22	23.03	0.44	21	23.30	0.53	23	23.14	0.60
6	20	22.94	0.58	17	23.46	0.41	23	23.21	0.47
7	21	23.16	0.55	15	22.96	0.83	22	23.33	0.37
8	22	22.90	0.82	24	23.17	0.51	21	23.23	0.51
9	22	22.89	0.47	21	23.02	0.50	22	23.17	0.47
10	19	22.97	0.52	21	23.10	0.39	23	23.01	0.80
11	16	23.04	0.45	21	23.27	0.41	23	23.38	0.41
12	21	22.80	0.45	24	23.14	0.70	21	22.91	0.86
13	21	22.91	0.46	22	23.05	0.44	22	23.31	0.54
14	21	23.04	0.42	19	23.10	0.52	18	23.58	0.46

[1]Mean blood leukocyte count measured in cells/ml and expressed on a $\log_2$ scale.

[2]Standard deviation of the mean.

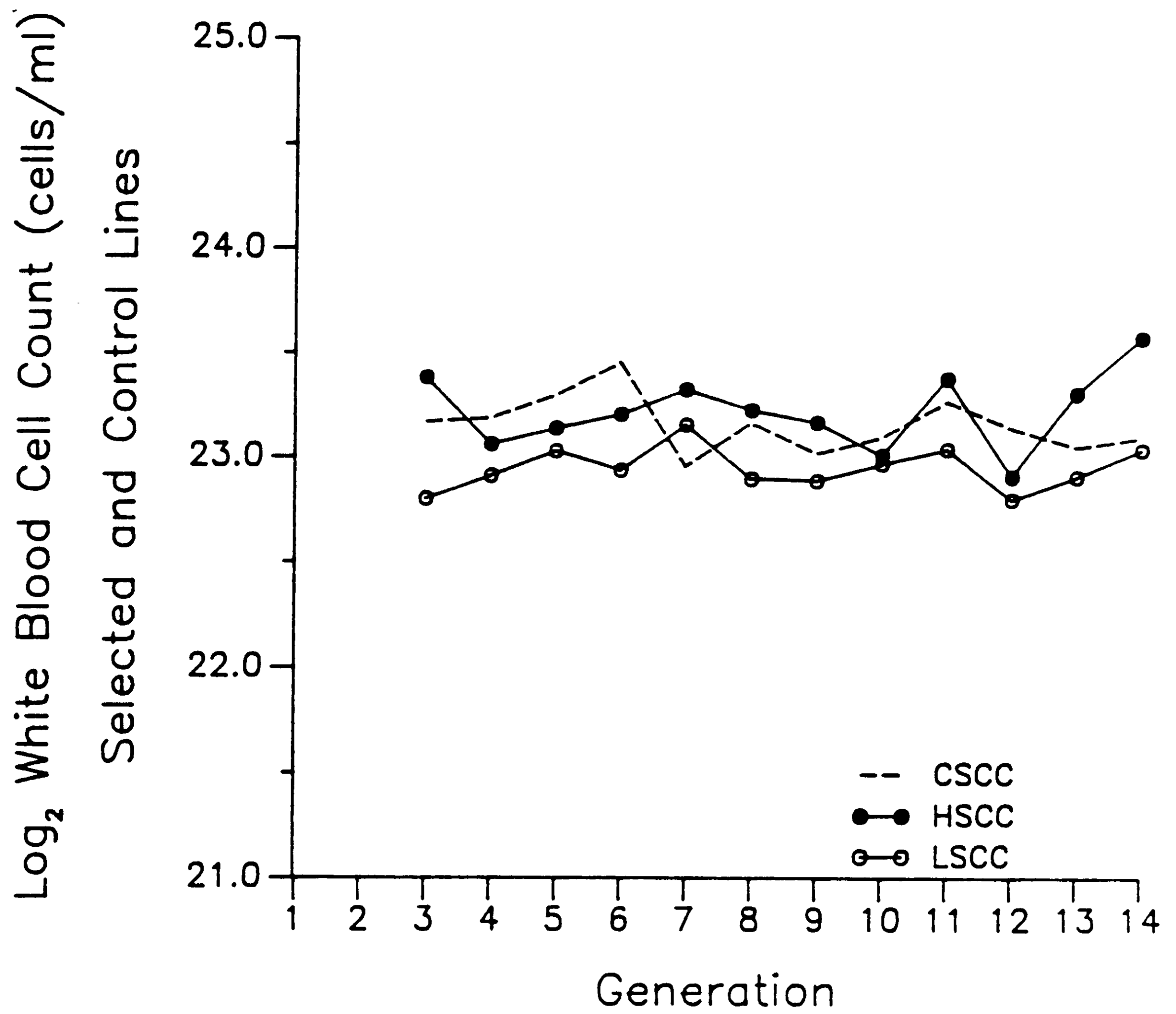

Figure 4. Change in $\log_2$ blood leukocyte count over time.

Percentage of Phagocytic Cells

Phagocytes play a key role in the defense of the mammary gland against infection. This is reflected in the cell composition of milk in which phagocytes predominate. It was of interest to discover if changes in milk SCC would be associated with changes in the level of phagocytes in the blood. Line generation means for the percentage of phagocytic cells (neutrophils and monocytes) in the blood of selected and control lines are in Table 9. The same information is graphically displayed in Figure 5. Generation means bounced erratically and all lines experienced an overall decline in percentage of phagocytic cells in the blood. The average response in the selected lines was -.2753 ± .3388 for the HSCC line and -.4185 ± .2260 for the LSCC line. The average change per generation in the control line was -.6364 ± .4670. None of the above responses were significantly different from zero (P > .05) and again, there was no significant diverenge between the selected lines (.1433 ± .2809; P > .05).

Table 9. Percentage of phagocytic cells (neutrophils and monocytes) in the blood of selected (LSCC, HSCC) and control (CSCC) lines.

	LSCC			CSCC			HSCC		
Generation	Number of Mice	Mean[1]	SD[2]	Number of Mice	Mean[1]	SD[2]	Number of Mice	Mean[1]	SD[2]
3	16	28.06	10.08	16	28.50	10.07	15	31.13	14.95
4	17	28.53	7.98	18	30.83	9.73	17	26.88	8.31
5	22	28.45	9.73	21	33.95	14.35	23	33.04	14.19
6	20	25.75	11.55	17	28.76	16.28	23	24.52	8.33
7	21	26.76	14.00	15	24.27	9.22	22	26.18	8.01
8	22	24.09	10.23	24	23.13	9.30	21	25.38	15.08
9	22	24.05	8.50	21	36.86	18.60	22	28.50	12.13
10	19	30.11	9.34	21	26.90	8.39	23	33.48	12.40
11	16	25.75	11.13	21	29.24	10.73	23	31.00	12.40
12	21	25.43	10.80	24	34.54	11.54	21	32.38	9.61
13	21	18.52	10.16	22	21.73	12.11	22	22.41	14.97
14	21	26.38	15.34	19	16.84	8.70	18	22.83	16.57

[1]Mean percentage of total white blood cell count which consists of phagocytic cells.

[2]Standard deviation of the mean.

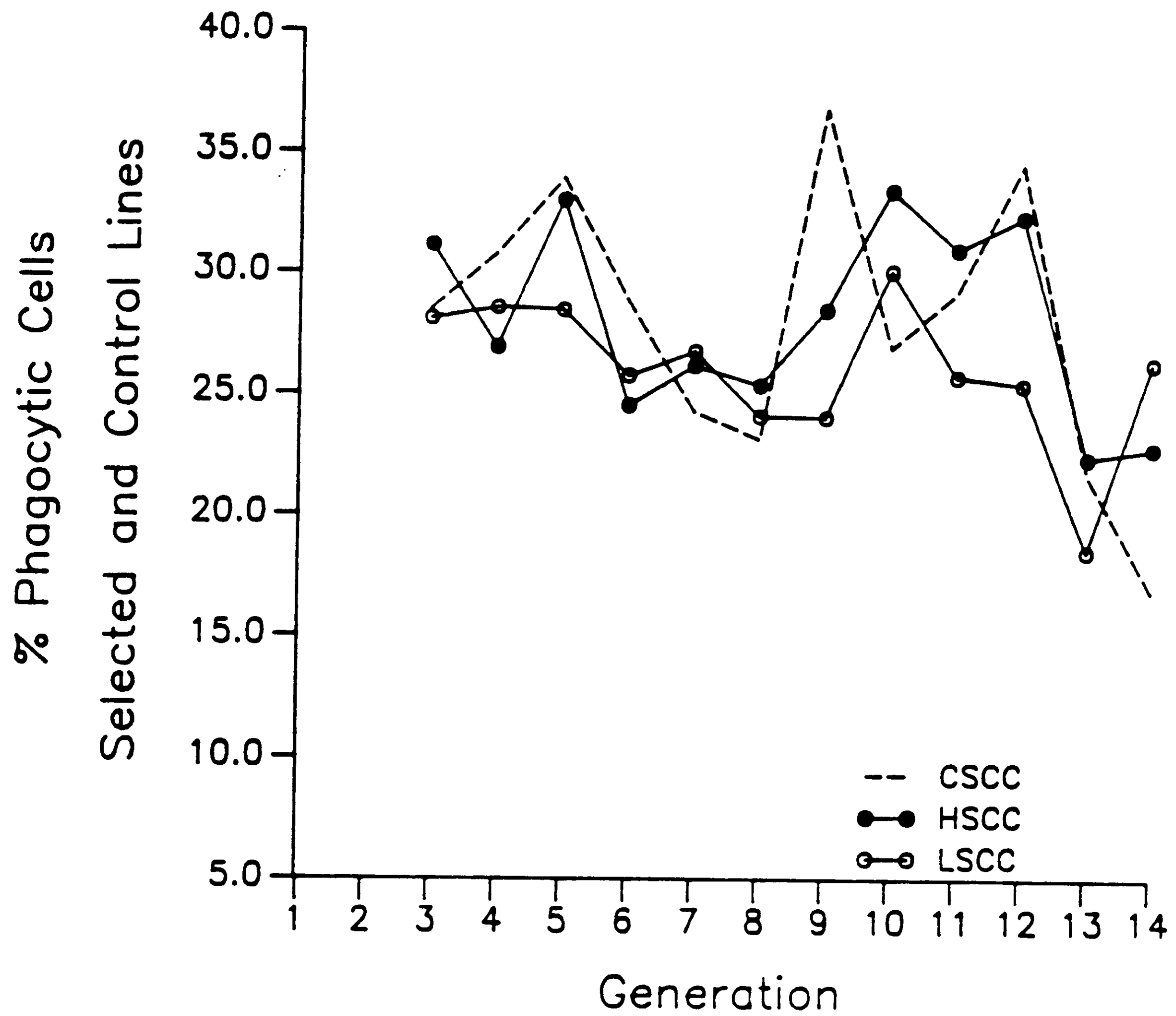

Figure 5. **Change over time in the percentage of phagocytic cells (neutrophils and monocytes) in blood.**

Response to Escherichia Coli Endotoxin Challenge

Given that leukocytes are a primary defense mechanism of the mammary gland, it was important to determine whether or not changes in milk SCC resulting from selection would alter the ability to respond to an infection-like challenge of the mammary gland. Table 10 contains line generation means for response to endotoxin challenge in the selected and control lines. Figure 6 displays the line generation means graphically. Overall, response to endotoxin challenge increased slightly in all lines across generations. The average response in the selected lines was .3570 ± .1528 for the HSCC line and .3547 ± .1455 for the LSCC line. The average change per generation in the control line was .3860 ± .1403. All regression coefficients were significantly different from zero ($P < .05$). However, there was no divergence betweeen selected lines (.0023 ± .1137; $P > .05$). The increase in response to endotoxin challenge which occurred in all lines is most likely the result of environmental change. One factor which contributed to environmental change was that the person administering the challenge injection changed in generation 9.

Table 10. Somatic cell count (SCC) response to endotoxin challenge of selected (LSCC, HSCC) and control lines (CSCC).

	LSCC			CSCC			HSCC		
	Number of	Log_2 SCC		Number of	Log_2 SCC		Number of	Log_2 SCC	
Generation	Mice	Mean[1]	SD[2]	Mice	Mean[1]	SD[2]	Mice	Mean[1]	SD[2]
4	17	20.01	4.56	18	19.59	5.38	17	20.66	4.66
5	22	17.31	4.43	21	18.63	5.31	23	19.20	5.09
6	20	17.23	5.01	17	17.25	4.96	23	17.34	5.06
7	21	17.39	4.97	15	15.42	4.45	22	15.67	4.17
8	22	21.04	4.97	24	19.03	5.09	21	19.74	5.27
9	22	20.35	3.71	21	20.06	5.03	22	20.13	4.43
10	19	21.73	2.95	21	19.57	5.26	23	20.88	4.29
11	16	22.61	2.74	21	19.59	5.04	23	21.44	3.55
12	21	20.84	2.94	24	20.47	3.98	21	21.19	3.57
13	21	21.10	4.05	22	22.46	3.86	22	21.91	3.72
14	21	20.39	3.75	19	21.31	4.48	18	21.50	3.92

[1]Mean difference in SCC between the challenge day and previous non-challenge day (Log_2 of SCC measured in cells/ml).

[2]Standard deviation of the mean.

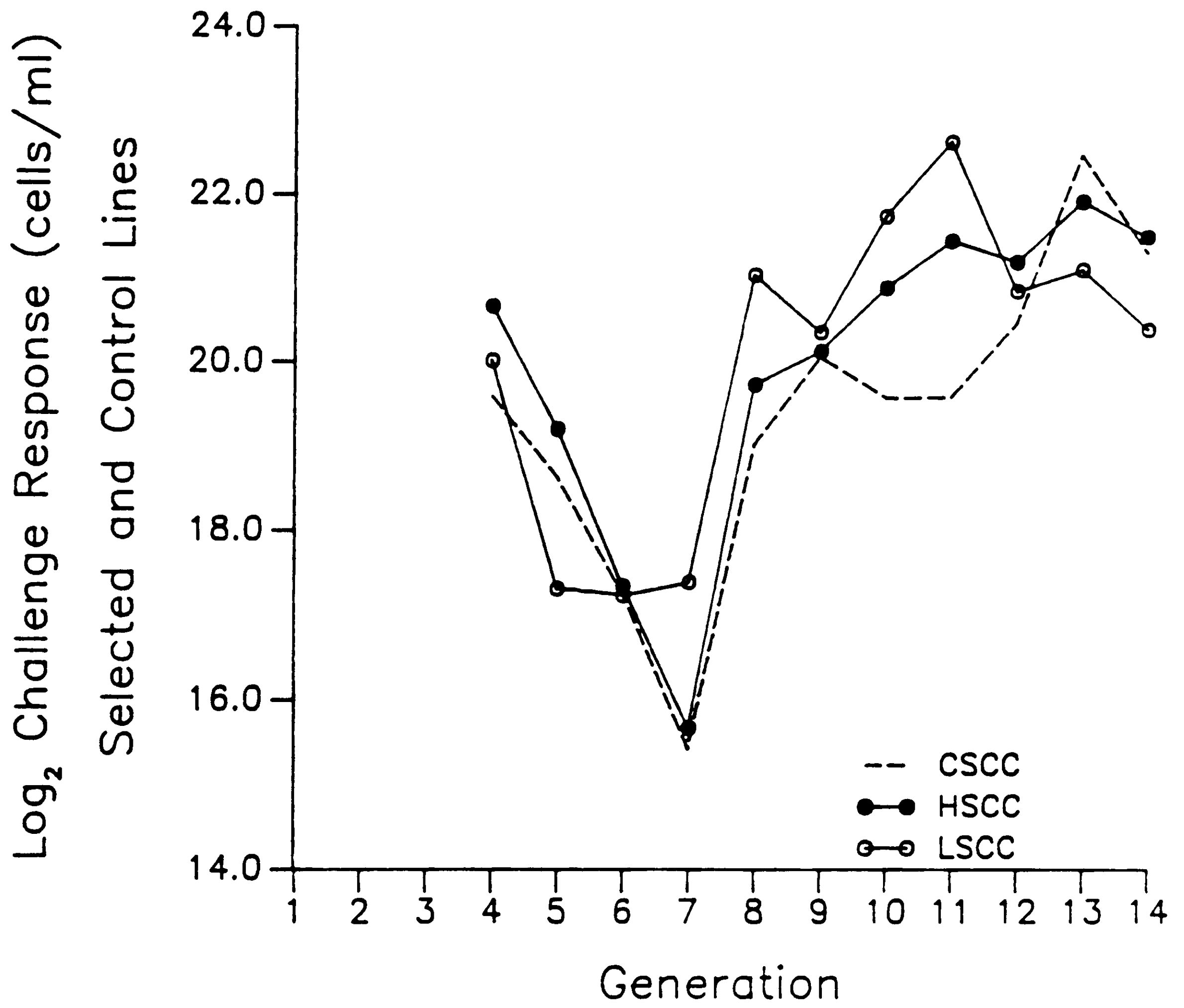

Figure 6. **Change in $\log_2$ response to endotoxin challenge over time.**

Percentage of Females Littering

Percentage of females littering is a measure of reproductive fitness. Reproductive fitness is an important element of any selection experiment, especially when the criterion of selection is related to a disease resistance mechanism of the animal. Line generation means for the percentage of females mated that eventually littered are in Table 11. These same means are also displayed graphically in Figure 7. The general trend for all lines was a decline in the percentage of females littering. Average response per generation was -.5341 ± .4725 in the HSCC line and -2.1407 ± .6275 in the LSCC line. The average change per generation in the control line was -.6374 ± .3871. Only the average response in the LSCC line was significantly different from zero ($P < .05$). The average divergence betweeen selected lines was also significantly different from zero (1.6066 ± .5231; $P < .05$). This divergence was the result of the more marked decline in the LSCC line. The percentage of females littering dropped sharply in the LSCC line for generations 8, 12 and 14 (figure 7).

Table 11. Percentage of females littering in selected (LSCC, HSCC) and control (CSCC) lines.

Generation	LSCC		CSCC		HSCC	
	Number of females exposed	Mean[1]	Number of females exposed	Mean[1]	Number of females exposed	Mean[1]
1	46	95	39	86	46	82
2	50	100	40	92	51	98
3	50	96	36	92	51	96
4	50	92	42	87	50	96
5	50	92	37	89	51	96
6	53	89	41	88	55	93
7	55	95	44	70	54	83
8	55	72	42	86	50	80
9	55	84	45	78	55	82
10	54	91	50	88	55	89
11	55	84	50	82	55	89
12	55	53	50	80	52	75
13	55	84	50	78	55	91
14	55	71	50	88	55	93

[1]Mean percentage of females mated that littered.

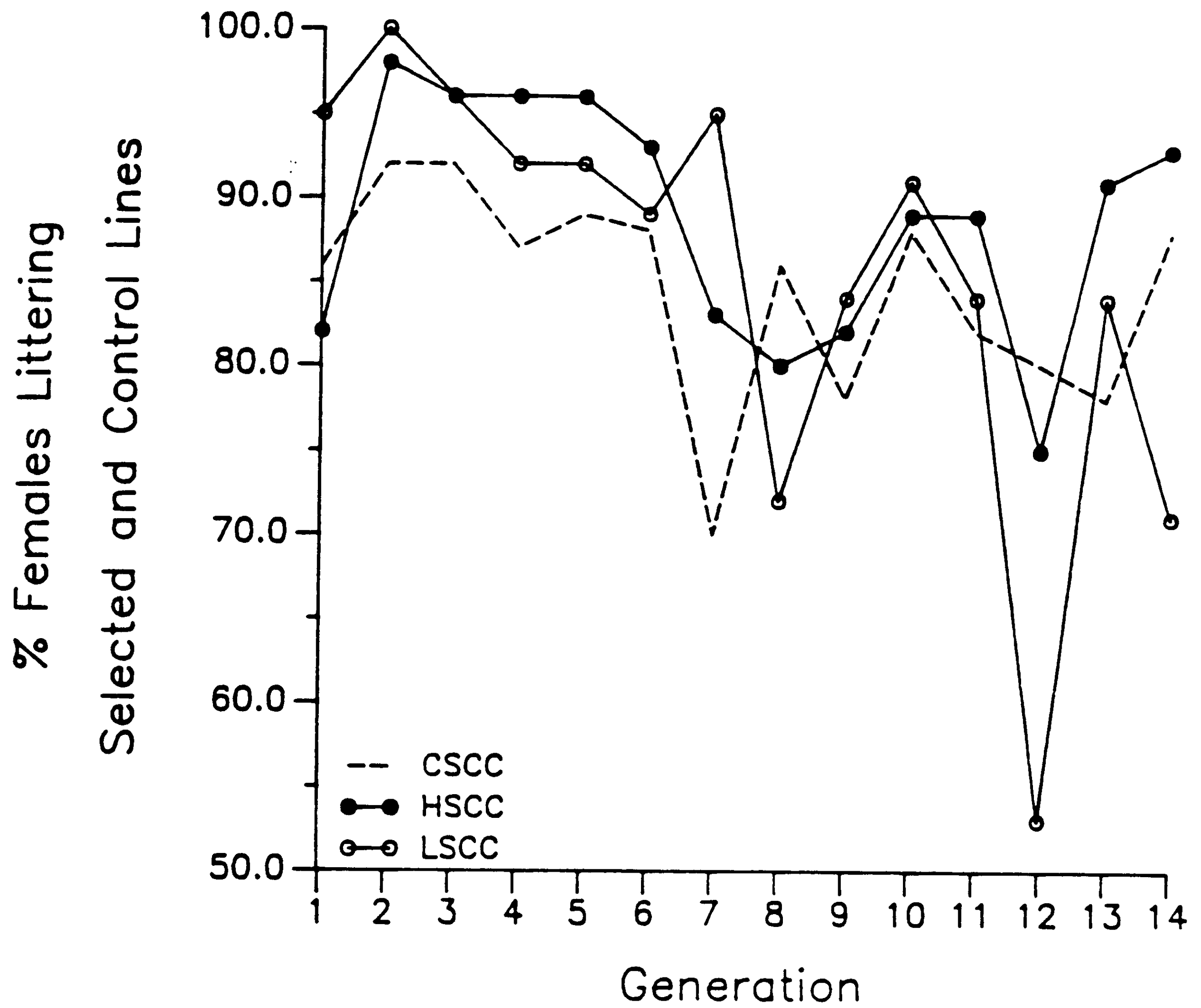

Figure 7. Change over time in the percentage of females mated which actually littered.

Number of Young Born Alive

As with percentage of females littering, the number of young born alive is also a measure of the reproductive fitness of the dam. Table 12 contains line generation means for the number of young born alive per litter in the selected and control lines. Figure 8 is a graphic display of this same information. As with the percentage of females littering, the number of young born alive declined across generations in all lines. Again, this decline was more marked in the LSCC line. Average response per generation in the selected lines was -.0290 ± .0370 for the HSCC line and -.1375 ± .0260 for the LSCC line. The average change per generation in the control line was -.0759 ± .0389. Only the regression coefficient for the LSCC line was significantly different from zero ($P < .05$). Average response measured as the regression of the difference between selected lines on generations was .1085 ± .0365. This response was also significantly different from zero ($P > .05$) and is a result of the more marked decline in the LSCC line.

Table 12. Line generation means for the number of young born alive in selected (LSCC, HSCC) and control (CSCC) lines.

	LSCC			CSCC			HSCC		
Generation	Number of Dams	Mean[1]	SD[2]	Number of Dams	Mean[1]	SD[2]	Number of Dams	Mean[1]	SD[2]
1	32	12.09	1.77	31	12.81	1.62	35	12.71	2.01
2	35	11.49	2.23	28	11.00	2.09	35	12.09	2.33
3	46	11.63	2.26	33	12.06	2.09	49	11.88	1.86
4	43	11.33	2.53	31	11.03	1.72	44	11.48	1.56
5	43	11.81	2.02	33	11.73	2.43	44	11.66	2.03
6	43	11.51	2.42	31	12.23	2.77	42	12.41	2.22
7	40	12.18	2.45	29	11.24	1.83	45	11.93	2.15
8	49	10.88	2.38	28	11.07	2.37	41	10.95	2.20
9	37	11.24	2.06	31	11.65	2.18	28	12.25	2.47
10	38	10.92	2.22	26	12.31	2.09	34	11.29	2.34
11	44	10.16	1.79	35	10.80	1.59	39	10.87	1.84
12	36	10.61	2.33	40	11.13	2.22	46	12.07	2.12
13	25	10.52	1.76	35	10.71	1.66	34	12.44	2.23
14	35	9.94	2.59	23	11.09	1.98	47	11.85	1.59

[1] Mean number of young born alive per dam.

[2] Standard deviation of the mean.

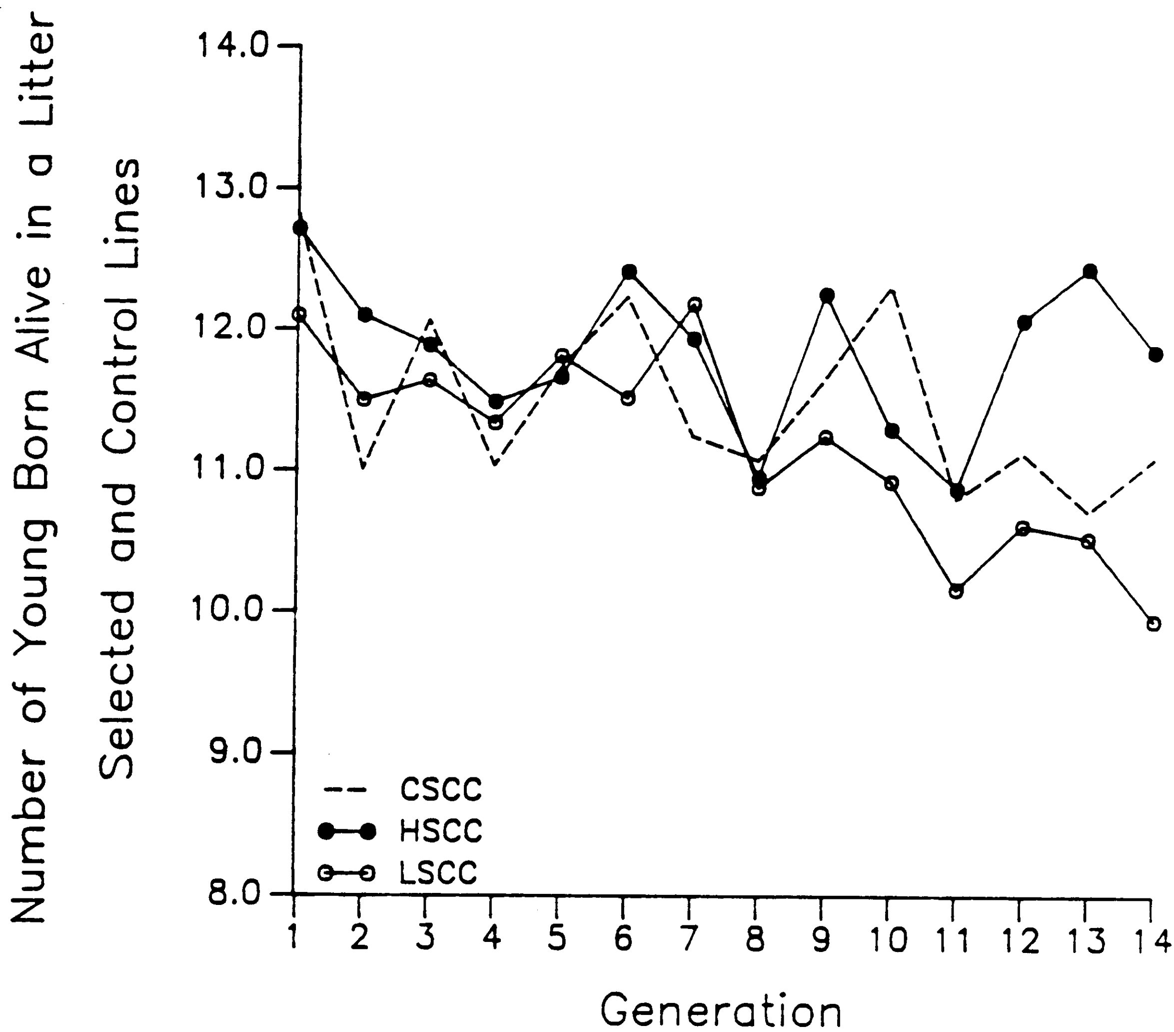

Figure 8. Change over time in the number of young born alive per litter.

Percentage of Young Surviving to Weaning

Percentage of young surviving to weaning measures both the maternal fitness of the dam and the fitness of the offspring. Line generation means for the percentage of young per litter that survive until weaning are listed in Table 13 and displayed graphically in Figure 9. The average response per generation for the number of young surviving to weaning was zero for both selected lines. The average change per generation in the control line was also essentially zero and not significant (-.0875 $\pm$ .0528; $P > .05$). As the generations progressed the range within which the means varied increased from one to two percent. There was no significant divergence between selected lines.

Table 13. Percentage of young surviving to weaning in selected (LSCC, HSCC) and control (CSCC) lines.

Generation	LSCC			CSCC			HSCC		
	Number of Dams	Mean[1]	SD[2]	Number of Dams	Mean[1]	SD[2]	Number of Dams	Mean[1]	SD[2]
1	35	99.43	2.36	28	99.64	1.89	35	99.14	2.84
2	47	99.57	2.04	33	100.00	0.00	50	100.00	0.00
3	44	99.77	1.51	31	99.68	1.80	44	100.00	0.00
4	43	100.00	0.00	33	98.79	3.31	45	99.56	2.98
5	43	98.84	3.24	33	99.09	3.84	45	98.67	4.57
6	41	99.51	2.18	29	98.28	4.68	46	99.13	3.54
7	49	99.80	1.43	28	100.00	0.00	42	99.52	2.16
8	38	99.74	1.62	32	98.13	4.71	30	100.00	0.00
9	38	100.00	0.00	27	98.15	5.57	35	97.71	5.47
10	45	98.44	5.20	35	97.71	5.98	41	99.27	2.63
11	36	99.72	1.67	40	98.50	5.33	46	99.78	1.47
12	25	100.00	0.00	35	100.00	0.00	34	99.41	2.39
13	35	99.71	1.69	24	97.92	5.88	48	99.17	2.79
14	34	99.41	2.39	33	99.70	1.74	40	100.00	0.00

[1]Mean percentage of young per dam, surviving to weaning.

[2]Standard deviation of the mean.

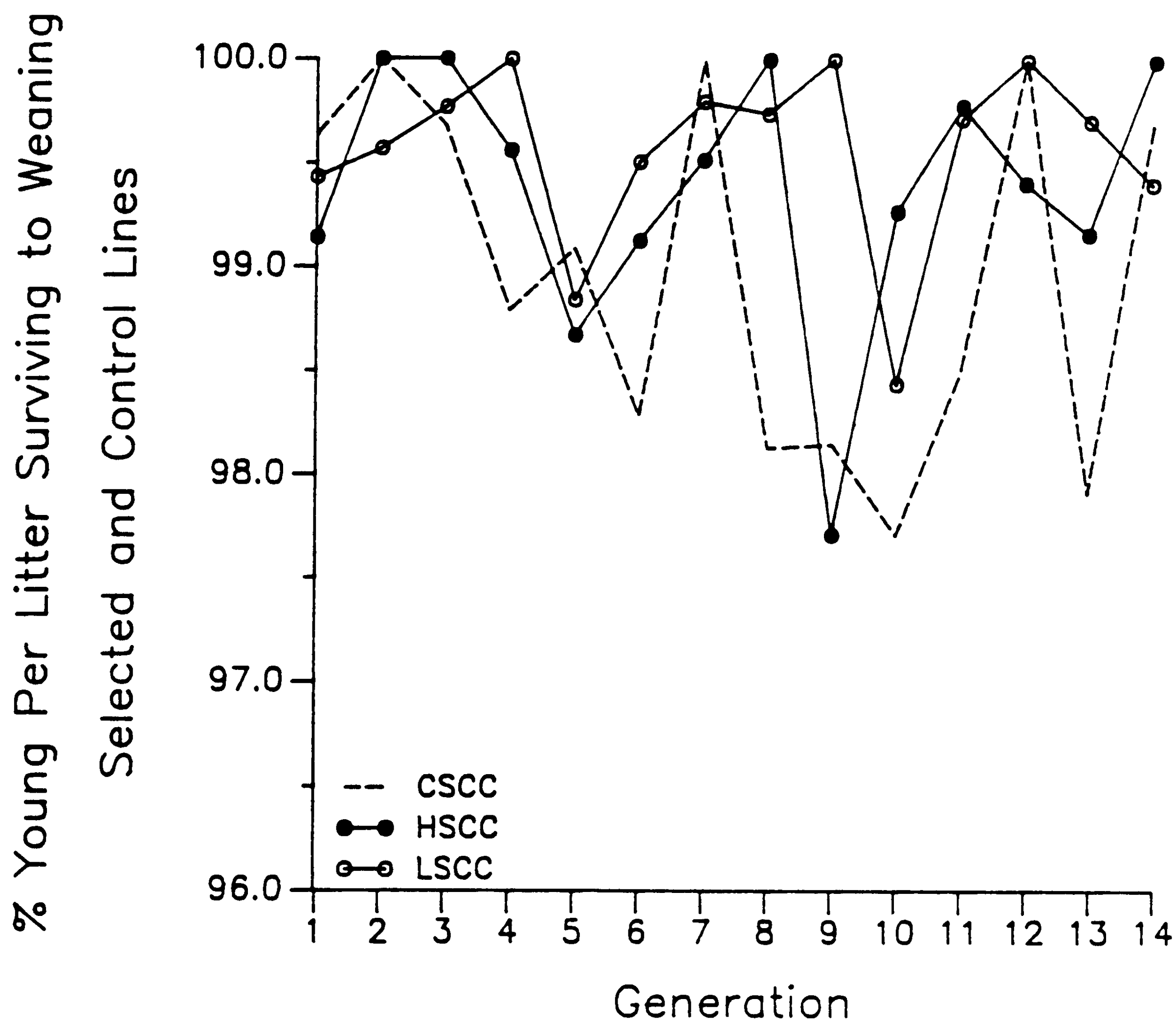

Figure 9. Change over time in the percentage of young per litter which survive to weaning.

Regression on Cumulated Genetic Selection Differential

Regression coefficients from the regression of HSCC - LSCC on the deviated (HSCC - LSCC) cumulative genetic selection differential are in Table 14. Given that an assumed heritability of .10 was already accounted for in the calculation of selection indexes, the regression of SCC on the cumulative genetic selection differential was expected to be 1.0 for true heritability of .10. However, the regression coefficient for SCC was much lower (.2253 ± .1134, $P > .05$) indicating that heritability may be lower than the assumed .1 used to calculate the selection indexes. Regression coefficients for percentage of females littering and number of young born alive were 9.5785 ± 3.7248 and .6099 ± .2662, respectively, and were significantly different from zero ($P < .05$). This implies that as the lines diverged genetically for SCC they also diverged with respect to reproductive fitness as measured by the percentage of females littering and the number of young born alive. However, because there is little evidence to indicate that the selected lines did diverge genetically with respect to SCC, the separation for reproductive fitness is most likely mediated by the decline in reproductive fitness in line LSCC which was previously described for percentage of females littering and number of young born alive.

Table 14. Coefficients and standard errors from the regression of performance on the cumulative genetic selection differential.

Trait	Coefficient[1]		S.E.
Milk SCC	.2253		.1134
Milk Yield	.3807		.5338
WBC Count	.0134		.1071
% Phag. Cells	.9274		2.0094
Chall. Response	-.3855		.8599
% Littering	9.5785	*	3.7248
Number Born	.6099	*	.2662
% Survival	-.0637		.3775

* Correlation coefficient significant $P < .05$.

[1] From the regression of deviated performance (HSCC - LSCC) on the deviated cumulative genetic selection differential.

Phenotypic Correlations

Phenotypic correlations among milk SCC, milk yield, white blood cell count, percentage of phagocytes in the blood, endotoxin challenge response, number of young born alive to a dam and the percentage of a dam's litter which survived to weaning, were computed within line, generation, mating group within generation and the line by generation interaction. Results are in Table 15.

Milk SCC

A small negative correlation (-.07, $P < .05$) was found between $\log_2$ lactation average milk SCC and lactation milk yield. This negative correlation agrees with the negative correlation reported in dairy cattle. Table 1 summarizes estimates of the phenotypic relationship between milk SCC and milk yield in dairy cattle from a number of studies. Estimates of the phenotypic correlation ranged from approximately zero to -.17 in dairy cattle. The small negative correlation in mice falls within this range.

A negative correlation (-.17, $P < .05$) was also found between milk SCC and endotoxin challenge response. This implies that increasing leukocyte levels in milk are associated with a slight decline in the ability of the mammary gland to respond to an infection like challenge by further increasing the number of available leukocytes. Perhaps there exists a physiological maximum for leukocyte diapedesis in the mammary gland. Individuals with higher SCC's would be nearer the maximum and have less room to increase the leukocyte response.

Phenotypic correlations between milk SCC and total white blood cell count, percentage of phagocytic cells in blood, number of young born alive, and percentage of young surviving to weaning were not significantly different from zero ($P > .05$).

Table 15. Phenotypic correlations among measured traits.

Trait	Milk Yield	WBC Count	% Phagocytic Cells	Challenge Response	Number Born	% Survival[1]
Milk SCC[2]	-.07 * (996)	-.06 (644)	-.01 (644)	-.17 * (600)	.00 (1554)	-.04 (1554)
Milk Yield[3]		.11 * (391)	-.02 (391)	.08 (359)	.08 * (1002)	.09 * (1002)
WBC Count[4]			.17 * (648)	.06 (604)	.06 (648)	-.01 (648)
% Phagocytic Cells[5]				.01 (604)	.07 (648)	.01 (648)
Challenge Response[6]					.04 (604)	-.03 (604)
Number Born[7]						.05 * (1562)

* Correlation coefficient significant $P < .05$.

[1]Percentage of young surviving to weaning.
[2]Log_2 of lactation average milk somatic cell count (SCC), adjusted for stage of lactation.
[3]Lactation milk yield.
[4]Log_2 of total white blood cell (WBC) count.
[5]Percentage of phagocytic cells (neutrophils and monocytes) in blood.
[6]Response to endotoxin challenge.
[7]Number of young born in a litter.

Milk Yield

The phenotypic relationship between milk yield and total white blood cell count was small and positive (.11, $P < .05$). Dams which produced more milk also had slightly greater blood leukocyte levels or, conversely, lower producing dams had lower blood leukocyte levels. One possible explanation for this small positive relationship may be production level stress. Greater milk production may place more stress on the dam, stimulating the immune system to increase blood leukocyte levels. Correlation coefficients between milk yield and percentage of phagocytic cells in blood, and endotoxin challenge response were not significantly different from zero ($P > .05$).

Both number of young born alive and percentage of young surviving to weaning were positively correlated with milk yield, however, both correlations were small (.08 and .09, $P < .05$). Milk yield is a measure of maternal fitness and should be reflected in traits measured on the young which are influenced by maternal fitness.

White Blood Cell Count , Percentage of Phagocytes in Blood and Endotoxin Challenge Response

The phenotypic correlation between total white blood cell count and percentage of phagocytes in blood was positive and significantly different from zero (.17, $P < .05$). This relationship implies that as the size of the white blood cell population changes, the change in the percentage of phagocytic cells is not proportional to the change in the total cell population, but it is in the same direction. Presumably differences in blood leukocyte levels are related to the health of the mice. The need for phagocytic leukocytes will increase during infection, disease or injury. Therefore it is not surprising that the proportion of phagocytic leukocytes in blood should rise and fall with the total blood leukocyte count.

Correlations between total white blood cell count and endotoxin challenge response, number of young born alive, and percentage of young surviving to weaning were not significantly different from zero ($P > .05$). Similarly, percentage of phagocytic cells was not significantly correlated with endotoxin challenge response, number of young born alive, or percentage of young surviving to weaning. Additionally, there was no correlation between challenge response and number of young born alive, or percentage of young surviving to weaning.

Number of Young Born Alive

Even with standardization, there was a small positive correlation between number of young born alive and percentage of young surviving to weaning (.05, $P < .05$). Both traits measure to some extent maternal fitness and would be expected to be positively related. However, because litters were standardized, the validity of this correlation both in size and sign is questionable.

Components of Genetic Variance for Milk SCC

Due to the lack of response to selection for milk SCC, the components of genetic variance for milk SCC were investigated. All 3 lines of mice (LSCC, HSCC, CSCC) were used to obtain estimates of the covariance between daughter and dam, and among full sibs. Lactation average $\log_2$ SCC's of daughters were regressed on those of the dam after accounting for nested effects line, generation and mating group (of daughter's birth). To estimate the covariance among full sibs, an analysis of variance was performed on lactation average $\log_2$ SCC. The model included nested fixed effects line, generation, mating group (of litter's birth) and litter. The covariance among full sibs was obtained from the variance among litters.

Degrees of freedom, mean squares and covariance estimates from the regression and full sib analyses are in Table 16. Covariance estimates from both analyses were negative (-.1180 from regression

of daughter on dam and -.0362 from full sibs). The covariance among full sibs is obtained from the litter component of variance which theoretically can not be negative. One possible explanation for the negative covariance between daughter and dam would be a negative covariance between the additive effect for the offspring component of SCC, and the additive maternal effect for SCC as expressed in the offspring. Consideration of such a covariance assumes that the character milk SCC can be broken down into 2 component characters, the offspring component and a maternal component (commonly referred to as a maternal effect). If a negative covariance between the additive effect of the offspring component and the additive maternal effect does exist, it would, for example, effect selection for high SCC in the following manner: offspring from a dam with a high SCC should also have the genetic makeup for a high SCC, however, these offspring will not be selected to be parents because the maternal effect of their dam will cause them to exhibit a low SCC when they in turn become dams.

The possibility of a negative covariance between the additive effect for the offspring component and the additive maternal effect for SCC was investigated. Estimates of the covariance between daughter and dam, among full sibs, and between granddaughter and paternal granddam were used to solve the equations below for the following offspring and maternal components for SCC: additive variance for the offspring component, additive variance for the maternal effect, and the covariance between the offspring additive effect and the maternal additive effect. The covariance among full sibs was set equal to zero because of the negative estimate obtained which is theoretically impossible given the method of estimation.

$$\text{Cov(Daughter, Dam)} = \frac{1}{2}\sigma^2_{Ao} + \frac{5}{4}\sigma_{AoAm} + \frac{1}{2}\sigma^2_{Am}$$

$$\text{Cov(Full Sibs)} = \frac{1}{2}\sigma^2_{Ao} + \sigma_{AoAm} + \sigma^2_{Am}$$

$$\text{Cov(Granddaughter, Paternal Granddam)} = \frac{1}{4}\sigma^2_{Ao} + \frac{1}{8}\sigma_{AoAm}$$

where:

Table 16. Degrees of freedom, mean squares and covariance estimates for analyses to obtain covariances between relatives.

	Daughter-Dam[1]		Granddaughter-PGD[2]		Full Sibs[3]	
	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Line	2	38.6886	2	36.7061	2	38.6886
Gen/Line	36	14.3139	39	6.1337	36	14.3139
Matgp[4]/Gen/Line	77	1.4537	84	2.2463	77	1.4537
Litter/Matgp/Gen/Line			738	1.7714	498	1.6505
Error	1345	1.7021	439	1.8949	848	1.7342
Relative Covariance		-.1180		-.0067		-.0362

[1]From regression of daughter on dam.

[2]From regression of granddaughter on paternal granddam (PGD).

[3]From analysis of variance for full sibs.

[4]Mating group.

σ^2_{Ao} = additive component of variance for the offspring component.

σ^2_{Am} = additive component of variance for the maternal effect.

σ_{AoAm} = covariance between the additive effects for the offspring and maternal components.

Equations for the coavariances among relatives were taken from Willham (76). The covariance between granddaughter and paternal granddam was obtained from the regression of granddaughter on paternal granddam after accounting for nested effects line, generation, mating group (of granddaughter's birth), and litter (of granddaughter's birth). Degrees of freedom, mean squares and the covariance estimate from this analysis are in Table 16. As with the covariance between daughter and dam, the covariance between granddaughter and paternal granddam was also negative (-.0067).

The following values were obtained for the components of genetic variance:

$\sigma^2_{Ao} = .0622$

$\sigma^2_{Am} = .1470$

$\sigma_{AoAm} = -.1781$

This analysis indicates that there is a negative covariance between the additive offspring and maternal effects for milk SCC in mice. However, there is no apparent biological explanation for the dam's SCC should influence the performance of her offspring when they are grown. Heritability was calculated from the estimate of the additive component of variance for the offspring component for SCC. Total phenotypic variance was obtained from a within generation analysis of variance for SCC (1.3308). Heritability was .05. This is considerably smaller that the assumed .10 used in the selection index calculations if transmitting ability.

Conclusions

The results from this study imply that selection for milk SCC as a means of increasing genetic resistance to mastitis may not be feasible or effective. Genetic and phenotypic selection differentials indicated that selection methods did succeed in choosing the more extreme individuals to continue the selected lines. However, direct response to selection for lactation average $\log_2$ SCC was very low and does not appear to have created lines genetically divergent for milk SCC.

Average response per generation in the 7 secondary traits (milk yield, total white blood cell count, percentage of phagocytic cells in blood, endotoxin challenge response, percentage of females littering, number of young born alive, and percentage of young surviving to weaning) revealed no differences between the selected lines which could be attributed to selection for SCC. This was expected given the lack of direct response to selection for SCC. All lines experienced an overall decline in milk yield of approximately 2 to 3 grams. There were no changes in total white blood cell count, but a slight decline did occur in percentage of phagocytic cells in blood for all lines. Average change per generation in endotoxin challenge response increased for all lines. A small decline occurred in percentage of females littering and number of young born alive, with a more marked decline occurring in line LSCC for both traits. The percentage of young surviving to weaning did not change but the range in which line generation means varied increased from 1 to 2 percent.

Phenotypic correlations among SCC and the 7 secondary traits were generally small and near zero. They ranged from -.17 to .17. The phenotypic relationship between SCC and milk yield in mice was negative and within the range of correlations reported for dairy cattle. The correlation between milk SCC and endotoxin challenge response was also negative. Mice with higher SCC's were somewhat less able to respond to an infection like challenge of the mammary gland by increasing the number of available leukocytes within the gland. Correlations between milk yield and white blood cell count, number of young born alive, and percentage of young surviving to weaning were

small and positive. Total white blood cell count was positively correlated with percentage of phagocytic cells in blood, indicating that the proportion of phagocytic leukocytes in blood rises and falls with the total blood leukocyte count.

Examination of components of genetic variance for milk SCC yielded negative estimates for covariance between daughter and dam, and among full sibs. Based on the negative covariance estimate between daughter and dam, the possibility of a negative covariance between additive effects for the offspring component and maternal effect as expressed in the offspring, was investigated. This covariance was found to be negative and is one possible explanation for the lack of direct response to selection for SCC. Additionally, heritability was calculated from the estimate for the additive component of genetic variance fo the offspring component for SCC. The value of .05 obtained was much smaller than the assumed .10 used in the calculation of selection indexes and is another factor contributing to the lack of response to selection.

The discovery of a negative covariance between the additive effects for the offspring component and maternal effect for SCC was surprising because there is no readily apparent biological explanation for its existence. If such a relationship exists in dairy cattle it is likely to be of little practical significance because offspring are generally raised apart from their dams.

The results of this study are not encouraging for the use of selection for SCC as a means to increase genetic resistance to mastitis in dairy cattle. However, further selection research in mice which takes into account the negative covariance between additive effects for the offspring component and maternal effect for SCC, is needed to better assess the usefulness of selection for SCC.

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