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The effects of early life exposure to differential housing on adult immunocompetence in male and female Sprague-Dawley rats

Margaret A. Mihalczo

To the Graduate Council:

I am submitting herewith a thesis written by Margaret A. Mihalczo entitled "The effects of early life exposure to differential housing on adult immunocompetence in male and female Sprague-Dawley rats." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Psychology.

Debora Baldwin, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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THE EFFECTS OF EARLY LIFE EXPOSURE TO DIFFERENTIAL HOUSING ON ADULT IMMUNOCOMPETENCE IN MALE AND FEMALE SPRAGUE-DAWLEY RATS

A Thesis

Presented for the

Master of Arts

Degree

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Margaret A. Mihalczo

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ABSTRACT

The purpose of this study was to determine the influence of early life housing conditions on adult immunocompetence (antibody formation, relative spleen weight, and T-cell count) and peripheral blood glucose levels. Eighteen young (44 days of age) male and female Sprague-Dawley (SD) rats were randomly assigned to one of the 3 housing treatment conditions (1, 3, or 6/cage) and segregated by sex. At the end of 36 days, all animals were injected with 1 ml of a 10% suspension of sheep red blood cells (SRBC). Seven days after immunization, the animals were sacrificed. The results were mixed with regard to the immune parameters investigated. Individually housed animals displayed significantly depressed antibody response when compared to the animals housed 6/cage. There was no main effect of gender on antibody formation. However, male and female animals did differ significantly with regard to peripheral blood glucose level, relative spleen weight, and body weight. The findings from this investigation may suggest that exposure to a presumably stressful event early in life may alter adult host resistance.

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CHAPTER I

INTRODUCTION

The effects of early life experiences have been studied from behavioral and physiological perspectives for many years. Isolation or individual housing has been one of the various stressors employed in studies of early life experiences. Researchers have studied the effects of isolation on dogs, monkeys, rats, and humans. Studies such as these were attempts to examine a possible relationship between the stressor of early isolation and later susceptibility to disease. It has been suggested that early life experiences can affect the development of neuroendocrine control mechanisms (Ader, 1970), thus influencing subsequent resistance to disease. In addition, few studies have specifically looked for possible sex differences. It is the purpose of this study to attempt to determine the influence of early life housing conditions on measures of immunocompetence (antibody formation, spleen weight and T-cell count), and whole blood glucose levels.

Definitions of Stress

Stress is a term used every day in both popular and scientific language. However, there appears to be no generally agreed upon definition, as most researchers are quick to note.

Hans Selye is considered to be the "father" of stress research. He has defined stress as "the nonspecific response of the body to any demand placed upon it" (Selye, 1973, p. 62). More specifically, the nonspecific response occurs with mental as well as physical demands. In a similar vein, Asterita (1985) has expanded Selye's response definition of stress stating that the physiological responses remain elevated even when the challenge is removed.

Because no single definition of stress has generally been agreed upon, Engel (1985) has suggested that the concept is untestable and lacks internal consistency. He argues that the term should be abandoned altogether. However, researchers have suggested that the term can be used in reference to, but not limited to, (1) exposure to objectively or normatively threatening, aversive, or demanding conditions; (2) individual appraisals regarding threat or demand, and (3) physiologic changes (Newberry, Baldwin, Madden, & Gerstenberger, 1987).

Concepts of Stress

Cannon (1935) described a new concept which he called homeostasis. He believed in order for an organism to maintain health, nothing in the body must be allowed to deviate far from the norm. According to Cannon, if physiological norms are not maintained, illness or death can result. Thus, homeostasis was defined as the ability of the body to maintain normal physiological processes. In other words, homeostasis was necessary to maintain health.

Although Cannon was mainly concerned with studying the physiological basis of homeostasis, he also was interested in studying the influence of the sympathetic nervous system on internal secretions, of the endocrine system on metabolism, and of emotional disturbances on various physiological processes. His research on the sympathetic nervous system led to what is called the "fight or flight" response. The main purpose of this response is to protect the organism and guarantee its survival in the face of danger. Such an emergency response involves the activation of the sympathetic portion of the autonomic nervous system and the activation of the adrenal medullary axis. Cannon's research demonstrated the release of catecholamines into the bloodstream causing physiological arousal in response to a dangerous situation. These catecholamines are then used to prepare
the body for the "fight or flight" situation. Because of this finding, Cannon's research has been credited with leading to the discovery of a broad series of neuroendocrine responses to psychosocial stimulation (Henry & Stephens, 1977).

Selye (1946) noted not the differences between illnesses, but instead the sameness. He noted the loss of weight, generalized aches and pains, inflammation, and the loss of energy in patients who suffered from very diverse illnesses. Selye later called this observation "biologic stress." Ten years later, Selye discovered a syndrome that caused him to explore "biologic stress." He observed an enlargement and hyperactivity of the adrenal cortex, atrophy of the thymus gland and lymph nodes, and the appearance of gastrointestinal

ulcers, effects produced by a variety of impure and toxic gland preparations. These observations led to the development of the concept of the general adaptation syndrome (GAS), which was seen as an attempt by a threatened organism to adapt to the demand. Selye further explained the syndrome was "nonspecific" and represented a physiological response to damage as diverse as exposure to cold, surgical injury, production of spinal shock, excessive muscular exercise, or intoxications with sublethal doses of drugs. He described the syndrome as being made up of 3 stages: (1) alarm reaction or the "general call to arms" of the body's defensive forces, (2) stage of resistance or the organism's full adaptation to the stressor, and (3) stage of exhaustion which occurs if the stressor is sufficiently severe and prolonged.

In conclusion, Selye has emphasized the fact that stress is part of life and cannot be avoided. He stated there are four basic variations of stress: (1) good stress or eustress, (2) distress or harmful stress, (3) overstress or hyperstress, and (4) understress or hypostress. Selye (1946) emphasized that an individuals' goal should be to find as much eustress as possible and to minimize distress.

Mason (1971) has disagreed with Selye's concept of nonspecificity of the stress response. He argues that all stressful situations share an element of emotional stress. According to Mason (1971), it is the emotional reaction of the organism that creates the illusion of a generalized response rather than the physical demand. Mason's (1971) first experiments on the effects of stress on the endocrine system employed the use of exercise, fasting, and

temperature as stimuli. For example, in his first study on fasting, 2 out of 8 monkeys were simply deprived of food for 3 days, but remained housed in the same environment as the monkeys who were not deprived of food. A marked increase in urinary 17-hydroxycorticosteroid (17-OHCS), an index of stress, was found in the deprived monkeys compared with the monkeys in the control group. In an attempt to determine if this elevation in corticosteroid was the result of physiological demands (food deprivation), a second study was designed to minimize possible psychological variables. The fasting monkeys were housed separately from the nondeprived monkeys. The researchers found no significant increases in 17-OHCS response to fasting between the groups. Mason (1971) concluded that fasting itself elicited very little adrenal cortical response. More specifically, the elevation in the stress hormone was due to the psychological arousal as opposed to a physical demand.

Lazarus is another researcher who has disagreed with the nonspecific concept. According to Lazarus, (1981) cognitive variables affect the interpretation of stressful events. He has postulated that it is neither the environmental event nor the person's response that define stress, but rather the perception of the individual. According to Lazarus (1981), stress is the consequence of appraisal, not its antecedent. In other words, one's view of a situation can cause an event to be stressful. Lazarus and Folkman (1984) have described psychological stress as resulting from a particular relationship between the person and the environment that the individual appraises as being beyond his or her resources, thus endangering well-being.

In an effort to examine the effects of an individual's perception to stressful stimuli on physiological parameters, Lazarus, Opton, Markellos, and Rankin (1965) designed an experiment that employed the use of a stress-inducing film. Sixty-six college students were randomly assigned to three experimental conditions; each condition contained half male and half female subjects. The three experimental conditions were defined by three tape-recorded orientation passages which the subjects heard before viewing the motion picture. The film portrayed various woodshop working accidents. The passages were based on the concepts of denial, intellectualization, and the third, a control condition. The denial passage stated that the "employees" were actually actors and the accidents were faked. The intellectualization passage informed the viewer that the film was used to train employees about on-the-job safety. The passage suggested the film would be interesting to analyze from a socio-psychological perspective. Finally, the control passage simply gave the facts regarding the disturbing content but suggested no particular method of defensive coping as with the other conditions. The researchers reported the subjects in the control condition showed the highest skin conductance and heart rates which indicated the highest level of stress of the 3 groups. They concluded that the orienting passages acted to either increase or decrease subjects' interpretations (appraisal) of the events in the stimulus film, and thus were crucial in determining the "stressfulness" of the event.

Type of Stressors

Stressors may be either physical, psychological, or psychosocial. Physical stressors may be environmental pollutants, immobilization, electric shock, a decrease in oxygen supply, prolonged exercise, hypoglycemia, injuries, or other trauma to the body (Ader & Cohen, 1981). Psychological stressors, on the other hand, result from an individual's thoughts or feelings about real or imagined threats (Asterita, 1985), such as the loss of a loved one, depression, or test anxiety. Psychosocial stress has been described as a personal reaction to an intense social situation (Asterita, 1985). Some investigators have described psychosocial stress in terms of differential housing (isolation), light-shock stimulation, handling, or life changes. Others (Plaut & Friedman, 1981) describe this variable in conceptual terms, such as fighting, crowding, experimental anxiety, or novelty.

Even though stressors are classified as physical, psychological, and/or psychosocial, most researchers recognize that in real life it is difficult to separate the three types. For example, Rogers, Dubey, and Reich (1979) have noted that the physical stressor of starvation is accompanied by the psychological stress of hunger and fear of dying. The difficulty in measuring a response due to a stressor is that there is no direct way of knowing the organism's inner psychological experience. As Lazarus (1981) has pointed out, an individual's perception of an environmental event is paramount to the stress response.

General Immunology

Myrin Borysenko (1987) has written one of the most complete overviews of the immune system. The immune system is responsible for maintaining the integrity of an organism against foreign substances. Immune responses occur when opportunistic pathogenic microorganisms such as bacteria, viruses, and parasites invade the body. In addition, the immune system has the ability to protect the body from mutant cells that could develop into neoplasia or tumors.

According to Borysenko (1987) a hallmark of the immune system is the ability of the organism to distinguish between "self" and "nonself." Individuals can respond to any number of foreign substances called antigens. If previously presented antigens invade the body, they will be recognized by the immune system via the process of immunological memory.

The immune system can be divided into 2 major parts: cell-mediated immunity and humoral-immunity. The lymphocyte is the basic cellular unit of both types of immunity. There are 2 different types of lymphocytes, the T-lymphocyte and the B-lymphocyte. Although both types originate in the bone marrow, the T-lymphocyte is mainly involved in cell-mediated immunity, while the B-lymphocyte is mainly concerned with humoral immunity. The T-cells require the thymus and the B-cells the bursa-equivalent for maturation. In addition to the primary cells of immunity (B- & T-cells), a number of other cell types (e.g., monocytes, mast cells, macrophages, and natural killer cells)

contribute to the overwhelming complex process of immunosurveillance (Guyton, 1986).

In cell-mediated immunity, T-lymphocytes eliminate antigens either by attacking them directly or by releasing lymphokines which neutralize the foreign material when contact between the antigen and the T-cell is made. At this time, lysis of the antigen may occur, or aid the process of phagocytosis. Research has also indicated that certain T-cells (helper T-cells and suppressor T-cells) are responsible for the regulation of hormonal responsiveness to antigens (Jerne, 1976; Marx, 1985).

In humoral immunity, activated B-lymphocytes (plasma cells) secrete antibodies that circulate, combine with antigens and form inactive complexes. Antibodies or immunoglobulins (e.g., IgG, IgA, IgM, IgD, and IgE) are proteins found in the globulin fraction of the plasma. Each immunoglobulin unit is a Y shaped protein consisting of two long chains and two short chains of amino acids. At two ends of the Y shaped molecule are specific antigen binding sites. When an antigen encounters an immunoglobulin capable of binding to that antigen, the actual binding triggers an immune response which neutralizes the antigen. In addition, antibodies may destroy foreign material by activating macrophages and/or killer cell lymphocytes. Such a reaction is called antibody-dependent cytolysis (Borysenko, 1987).

Immunologists have developed various methods to assess the general level of immune function known as immunocompetence. Two of these methods are the in vivo technique, which measures antibody response to a

specific antigen, and the in vitro technique which uses nonspecific lymphocyte stimulants (mitogens), such as Phytohemagglutinin (PHA) and Concavalin A (Con A) (Schleifer, Scott, Stein, & Keller, 1986). These mitogens are used to measure the potential of lymphocytes to proliferate (Jessop, 1986). Thus, alterations in this ability may be indicative of changes in the ability of an animal to respond immunologically.

Stress and Disease

Selye (1973) stated that every disease causes a certain amount of stress, and stress plays some role in the development of every disease. Stressors can disrupt homeostasis in two ways, either by affecting the individual's ability to adapt to the situation or by causing disease due to a particular weakness in the structure of the organism (the vulnerable organ). According to Asterita (1985), stress-related disorders develop because of chronic elicitation of the stress response. Cumulative fight-or-flight responses can progress to specific end-organ dysfunctioning, pathophysiology, and possibly even death. The following section will describe studies that have examined the relationship between stress and disease.

Cancer

According to the immune surveillance theory, mutant cells normally are detected and destroyed by the body. If, however, the immune system is depressed, the cancerous cells are not detected and are allowed to proliferate

and develop into tumors. Research indicates that stress may be a factor in the suppression of this bodily system (Suter, 1986).

The role of the immune system as a mediating variable between cancer and stress is an area which is currently being studied. Riley, Fitzmaurice, and Spachman (1981) have reported various investigations which provide evidence that certain immune-related tumors are susceptible to the effects of stress in animal experiments. However, few human studies are available on stress-related variables and cancer.

Animal Studies

Dechambre and Gosse (1973) investigated the effects of individual versus group housing in female C57BL/6 mice with grafted tumors in a series of experiments. One group of animals was weaned at 21 days old, kept in cages in groups of 10 and injected with Krebs-2 ascites tumor cells at the age of 10 weeks. The animals were then returned to 10/cage or housed 1/cage. Another group of animals were housed individually at 21 days of age for 5 weeks and thus one-half of the animals were rehoused 10/cage while the others remained 1/cage. The authors reported the weight of ascites fluid and adrenal weights in the isolated mice exceeded that in the mice housed 10/cage but that the total number of tumor cells did not differ. They concluded that adrenal hyperactivity and increased ascitic fluid weight were related to an abrupt change in the environment (isolated vs. group) rather than the housing conditions themselves.

Newberry, Gildow, Wogan, and Reese (1976) conducted a series of experiments that demonstrated an inhibitory effect of stress on the development of 7, 12-dimethylbenz (a) anthracene (DMBA) induced rat mammary tumors in female Sprague-Dawley female rats. The animals were restrained in hardware cloth tubes which prevented them from turning around but allowed them to rotate about on their longitudinal axis. Two groups were restrained from light cycle onset to 5 or 10 hours later, from 35 to 108 days of age. The control group was not restrained. A dose of 0.4 ml of DMBA was administered by caudal vein injection at 50, 53, and 56 days of age to all animals. Animals were palpated for mammary tumors at 96, 102, and 108 days of age. They reported that the controls developed more tumors than did either restraint group but that the 2 restraint groups did not differ. The researchers concluded that chronically-administered restraint inhibited the development of DMBA-induced rat mammary tumors.

Finally, Cooley, Henry, and Stephens (1976) compared the effects of electric shock, immobilization, and differential housing in rats injected with DMBA. These researchers found electric shock and immobilization to inhibit tumor proliferation while group housing enhanced mammary tumor growth.

Some researchers have found stress to exacerbate tumor development (Amkraut & Soloman, 1972; Dechambre & Gosse, 1973; Lundy, Lovett, & Wolinsky, 1979) while others have found stress to inhibit tumor growth (Burchfield, Woods, & Matthews, 1979; Molomut, Lazerne, & Smith, 1963; Newberry, Frankie, Wogan, & Reese, 1976; Raskis, 1952). Sklar and Anisman

(1979) have proposed these contradictory findings may be attributable not only to the chronicity of the stress regimen but also to the availability of coping responses. In an extension of animal research findings to humans, Sklar and Anisman (1982) concluded that stress does not cause tumors. Rather, it can influence the course of tumor development "through its demonstrated influence on neurochemical, hormonal, and immunological functioning."

Human Studies

In humans, fewer studies are available on stress-related variables and cancer. Fox and Temoshok (1988) note that it is not surprising that research seeking a link between stress and cancer has been inconclusive. They suggest that if stress has an effect on cancer it is likely that stress alone does not increase cancer risk. They postulate that predisposing factors such as pre-existing physical conditions, personality variables, childhood experiences, and social resources are all variables that may determine how stressful events may affect a person at a particular time.

A study by Rogentine, Van Kammen, Fox, Docherty, Rosenblatt, Boyd, and Bunney (1979) attempted to examine psychological variables in patients diagnosed with malignant melanoma. The researchers administered the Recent Life Changes Questionnaire (RLCQ), the SCL-90R (a symptoms checklist), and the Locus of Control Questionnaire (an inventory of stressful events for the previous 2 years) within 1 week after surgery for melanoma. Patients rated the amount of adjustment needed to cope with their illness on a

scale of 1 to 100, with the resultant figure called the "melanoma adjustment score." Patients who reported that their disease required more adjustment, suggesting that it was a greater life stress, showed less relapse over 1 year. The researchers concluded it was possible that endocrine changes due to psychological factors (e.g., denial, repression) may have modulated immune responses that resulted in cancer susceptibility.

Another study which examined the stress/cancer relationship was conducted by Shekelle, Raynor, Ostfield, Garron, Bieliauskas, Liu, Maliza, and Paul (1981). These researchers analyzed data on 5,397 males, aged 40-55 years, who participated in the Western Electric Health Study. The subjects were given complete physical exams, medical and family histories were obtained, and the Minnesota Multiphasic Personality Inventory (MMPI) was administered to assess personality characteristics. A depression scale on the MMPI was used to measure the degree of clinical depression. Subjects who had the highest depression scores were found to have twice the risk of cancer over the next 17 years. The association persisted even after correction for other risk factors such as smoking, alcohol, family history of cancer, and occupational status were made. The researchers reported that death from cancer was not associated with scores on any other standard scale of the MMPI. They also reported the cancer-groups and the non-cancer groups differed significantly in mean value only on the depression scale, indicating that the association was with psychological depression and not psychological disturbance in general. The authors concluded that psychological depression

may be associated with impairment related to mechanisms for preventing the establishment and spread of malignant cells.

Autoimmune Disease

During fetal development, cells are instructed that "anything they recognize is 'self' and therefore to be left alone" (Dwyer, 1990, p. 33). However, in some individuals the normal control mechanisms that stop attacks on self do not work appropriately. It appears in these cases that helper T-cells may not recognize "self" thereby allowing other T- and B-cells to attack the body's tissues. The process by which "self" attacks "self" is called autoimmune disease. Animal models in relation to autoimmune diseases will be discussed in a later section on sex differences.

There appear to be two different groups of autoimmune diseases. In one group, specific organs tend to be targeted, such as in diabetes. In the other group, the immune system causes damage to the blood vessels, such as in systemic lupus erythematosus (SLE). Other autoimmune diseases include rheumatoid arthritis, thyroiditis, myasthenia gravis, ulcerative colitis, and Graves' Disease. The following section will focus on rheumatoid arthritis and SLE.

Rheumatoid arthritis. Rheumatoid arthritis is a chronic, systemic inflammatory disease with an insidious onset. The cause is not known but often appears to be triggered by a stressful situation (e.g., trauma, infection, emotional upset). The evidence for arthritis being a stress-related illness is

correlational. However, much research has been conducted to determine the effect of psychosocial factors on altering the parameters of this disease (Bieliauskas, 1982).

Rimon (1962) suggested that there are two subtypes of rheumatoid arthritics: (1) patients with a strong genetic predisposition who develop arthritis irrespective of environmental factors and (2) patients without a genetic factor who require severe stress to precipitate the illness. The researcher conducted interviews on 100 female outpatients diagnosed with rheumatoid arthritis. Control patients were selected from a general medical clinic and matched for age, sex, marital status and duration of somatic illness. Rimon reported a correlation was found between important conflict situations and the appearance of the first joint symptoms. The researcher suggested that severe stress could precipitate rheumatoid arthritis. However, because this study was retrospective, it would be difficult to determine if stress actually precipitated the illness.

Soloman and Moos (1964) compared 49 female arthritic patients and 53 of their healthy female family members on the MMPI. The researchers reported that patients who were found to have greater functional incapacity scored significantly higher on MMPI scales that reflected (a) physical symptoms; (b) depression, apathy, and lack of motivation; (c) general neurotic symptoms; and (d) problems of uncontrolled impulses (e.g., prejudice, ethnocentrism, and dependency). In addition, the researchers reported that life events also appeared to play a part in the later development of rheumatoid

arthritis. Most of the patients studied reported environmental stress of a long-term rather than short-term nature. Soloman and Moos (1964) have suggested that long-term stress may be related to the later development of rheumatoid arthritis.

A more recent study (Baker, 1982) investigated life events in female patients seen at a rheumatological clinic who were referred for psychiatric assessment. The researcher conducted a psychiatric interview which included an arthritis history, family history, pre-existing personality and previous illnesses, and specific questions which related to important life events in the year preceding the onset of arthritis. Control subjects were selected from randomly-selected, age-matched healthy women. Twelve patients as compared to 5 control subjects reported bad relationships with their mothers. Three of the patients and none of the control subjects described serious mental illness in their mothers. Twelve of the 15 patients and 3 of the control subjects reported life events which were of moderate or considerable long-term emotional threat. In 11 patients the interval between the life event and the onset of the illness was less than 3 months. The researcher concluded that severe emotional stress modified the immune system in such a way as to initiate the autoimmune response. As in the previous study, the retrospective nature of the interviews and self-reports would make drawing conclusions that stress is related to the precipitation or exacerbation of rheumatoid arthritis difficult.

Systemic Lupus Erythematosus. According to McClary, Meyer, and Weitzman (1955), systemic lupus erythematosus (SLE) is a disease which is sensitive to the psychological stress of loss. More specifically, they argue that tensions generated in the interpersonal conflict situation contributes to the tissue pathology of this disease. In 13 of 14 cases in their study, the perception of subjective symptoms of lupus was preceded by emotional stress. The stress events seen as particularly meaningful included the loss or threat of a significant personal relationship by illness, death, or divorce. The researchers reported that in 4 of the patients, the stress provoked an immediate intense reaction of anxiety and depression which was soon followed by the fever, malaise, and joint pains characteristic of lupus. The remaining 9 patients reported less depression but work was noted to be less satisfying while symptoms of weakness, fatigue, and joint pains were noted soon after the loss. The authors concluded that life situations in these patients appeared to revive feelings of early-life reaction to loss in the mother-child relationship, which led to a failure of coping efforts.

In an effort to investigate the effects of stress on individuals with SLE, Otto and MacKay (1967) interviewed 25 females and 3 males via the Eysenck Personality Inventory during remission of their disease. The researchers found that patients reported significantly more deprivation in childhood compared to the control group. In addition, the onset of SLE was preceded by psychological stress, with the most significant stressors related to interpersonal relationships.

As Bayles (1972) has stated, it is interesting to speculate that rheumatoid arthritis represents a psychosomatic disease. However, he has also cautioned that careful statistical analyses need to be included in future studies, as do long-term population prospective studies.

Stress and Immunity

Differential Housing

Ader (1969) has noted that an animal's resistance to disease could be affected by psychological factors such as differential housing and handling in infancy. The following section will attempt to examine the effects of differential housing on the immune system.

Plaut, Ader, Friedman, and Ritterson (1969) designed a series of studies to investigate the effects of group versus isolation housing on resistance to malaria, Plasmodium berghei. This particular pathogen was chosen because it had been found to be sensitive to differences in population sizes. Male and female Charles River CD-1 mice of approximately 60 days of age were housed either 5 or 1 per cage. The animals were injected intraperitoneally (i.p.) with parasitized cells of the KBG-173 strain of malaria. The dose was diluted in normal saline and was considered to be almost 100% fatal to this strain of mouse. Group-housed animals were found to be less resistant to malaria, surviving a mean of 9.5 days after injection, while the individually-housed survived 17.6 days. No significant differences were found between males and females.

Joasoo and MacKenzie (1976) investigated the effects of differential housing (isolation or overcrowding) on lymphocyte proliferation to mitogens. Male and female rats, housed for 5 weeks 1/cage (isolation), 10/cage (overcrowding), or 2/cage (control), were immunized with human thyroglobulin emulsified in complete Freund's adjuvant by two injections 12 days apart and killed 2 or 4 days later. Lymphocyte responsiveness to mitogen was then assessed. Neither isolation nor overcrowding had a consistent effect on the in vitro response of the splenocytes to the mitogen PHA. However, when the lymphocytes were stimulated by the antigen, human thyroglobulin, there were effects related to housing. The researchers reported stimulation was enhanced in cells from crowded, and decreased in cells from isolated females, as compared to normally-housed rats. However, with cells from male rats, a reduced response to the antigen was found, in both isolated and overcrowding conditions.

Edwards and Dean (1977) subjected Swiss-Webster albino mice to several experimental manipulations involving group housing (2, 10, 30, and 60 mice/cage) and immunization with .5 ml of typhoid/paratyphoid vaccine. Just prior to grouping, one-half of each group was immunized with .5 ml typhoid-paratyphoid vaccine. Seven days post-immunization, all mice were challenged i.p. with 1 ml Salmonella typhimirium. Antibody response and mortality served as the dependent measures. The results indicated that all vaccinated groups were resistant to the infection. In addition, with increased crowding, there was a significant reduction in humoral antibody reaction collapsed across

immunization conditions. Finally, there was a significant increase in susceptibility to the infection and mortality for nonimmunized mice.

A more recent study by Jessop, Gale, and Bayer (1988) investigated the effects of isolation on lymphocyte and corticosterone levels on Sprague-Dawley rats. They reported that individual housing of previously group-housed animals was found to result in time-dependent changes in mitogen-induced lymphocyte proliferation and plasma corticosterone concentrations. During the first week of isolation, both B-and T-cell lymphocyte responses were depressed. However, within 2 weeks an enhancement was observed. These researchers concluded that isolation was accompanied by a depressed adrenocortical activity and that the immunosuppression associated with short-term exposure was mediated by factors other than corticosterone. Jessop (1986) has noted the presence of several variables, such as sex, species, immune parameters measured, duration of exposure to the stressor, and type of immunological challenge (antigen or mitogen) which make it difficult to give a firm conclusion regarding the effect of isolation on the immune system.

Early Life Experiences and Immunocompetence

The effect of early life experiences on adult health continues to be a thriving research area ever since Breurer and Freud first proposed that psychologically "traumatic" experiences during childhood were the source of adult neuroses (Seitz, 1954).

Experiences early in life have been shown in both animals and humans to have immediate and long-term effects on behavior and health (Hofer, 1981). Several studies have also considered the possibility that early infantile stress might permanently affect the immune system (Schleifer, Scott, Stein, & Keller, 1986). However, Ader (1970) cautioned against concluding that early stimulation is consistently detrimental or beneficial to the organism. Instead, he suggested that researchers consider the kind of early experience and the kind of stress involved and not just the effect of early experience.

Handling

Some of the earliest studies to investigate the effects of early life experience on disease outcome used the stressor of handling. However, Levine (1957) handled rat pups in an attempt to determine if handling in the infantile period could increase resistance to stress. Twenty-seven male Sprague-Dawley Holtzman albino rats were handled from day 1 through day 20. Twenty-nine pups were not handled through the first 20 days. At this time, 20 rats each from the handled and nonhandled groups were injected i.p. with a 20% glucose solution. The animals were then placed in individual cages and deprived of food and water for 24 hours. The researcher reported that body weights for the handled animals were significantly greater than for the nonhandled animals. There were no significant differences in mortality rates. In addition, adrenal weights in the nonhandled animals were greater than the weights of the handled groups. However, both groups of stressed rats

(glucose injected) were found to have significantly greater adrenal weights. Levine (1957) concluded that the nonhandled animals were more profoundly affected by stress, and that the handled animals showed significantly less change in adrenal function.

Newton, Bly and McClary (1962) found that handling in the immediate post-weaning period appeared to be beneficial to longevity. Male and female albino rats had been implanted with Walker carcinoma 256 cells at 45 days of age. The handled rats survived significantly longer (a mean of 33.0 days) as compared to the nonhandled animals (a mean age of 28.7). They suggested that environmental influences on the developmental processes were more subtle than had been previously realized.

Soloman, Levine, and Kraft (1968) attempted to study the effects of early handling on adult immunocompetence. Fischer rats were handled for 3 minutes by being moved from their nest to a box and then returned to their nest daily, from infancy until weaning. After this time, handled and unhandled animals were housed in separate cages 2-3 animals/cage. At 9 weeks all animals were injected i.p. with 100 mg of flagellin polymer, known to produce a vigorous antibody response in the Fischer rat. Animals were bled from the tail at 4, 7, 14, 21, and 28 days after primary immunization. On day 28, a booster of 100 mg of flagellin was given. Blood was drawn 7, 14, and 21 days after secondary immunization. No statistically significant antibody response was found between male and female animals. However, both overall primary and secondary antibody responses were significantly greater in the handled than in

the unhandled animals. The researchers concluded that the immunological responsivity of the adult appeared to be modified by early experience possibly by environmentally responsive hormones affecting thymic function.

Separation

Another type of early life experience which has been investigated is separation. Ader and Friedman (1964) separated Sprague-Dawley rats from their mothers at 15 days of age. Controls remained with their mothers until the usual time of weaning. At 45 days of age, all animals were inoculated with a suspension of Walker 256 tumor cells. The mortality rate in the early separated animals was found to be significantly greater than in the controls. The median survival time following transplantation for the experimental group was 21 days, compared to a median of 25 days survival time for the controls. No sex differences were found. The authors concluded that susceptibility to disease appeared to involve an interaction between the pathogen and environmental factors which could cause irreversible psychophysiological changes in the affected organism.

A more recent study by Reite, Harbeck, and Hoffman (1981) investigated the effects of separation on immunocompetence in adulthood in a pair of infant pigtailed monkeys. The monkeys had been raised together as peers since early infancy. The animals were separated at the age of 27 weeks for 11 days and then reunited. Weekly blood samples were obtained beginning in the 24th week. The mitogens concanavalin A (Con A) and PHA

were used to assess the ability of the lymphocytes to undergo blast transformation. The researchers reported a depression of lymphocyte stimulation by both PHA and Con A during the latter part of the separation and early reunion in both infants. They concluded that disruption of an attachment bond by separation may impair cellular immune function and could be related to the increase in morbidity and mortality seen after bereavement.

Michaut and Dechambre (1981) also studied the effects of separation in infancy on adult immunocompetence but used newborn outbred SW mice as subjects. Fifty-six control mice were permanently kept with their mother until weaning, while 52 experimental mice were separated from their mothers for 4 hours/day for the first week and 8 hours/day for the second week. On the 15th day, the mother was withdrawn completely from the experimental group. After weaning, all mice were separated according to sex. At 7-8 weeks of age, mice were immunologically challenged with sheep red blood cells (SRBC). There was a significant difference between groups on the plaque forming cells (PFC) assay which measured antibody proliferation. Animals which were separated from their mothers displayed a decrease in the number of plaques compared to the control animals. The authors concluded that the early experience of maternal deprivation resulted in lowered sensory stimulation and provoked some abnormalities in development which later led to decreased adult immunocompetence.

Human Study

Finally, Lewis, Thomas, and Worobey (1990) studied the infant's ability to cope with stress. They hypothesized that infants who were less able to cope with stress would show a more pronounced response in stressful situations and might be at greater risk for health disorders related to the immune system. To test this hypothesis, they observed the infant's ability to suppress responding to an acutely painful stimulus (a heel lance to check for hereditary and metabolic disorders) at birth and at 2 months of age. The dependent variables were health outcomes (e.g., atopic disorders and infections) reported at 18 to 24 months. No relationship was found between the child's response at 2 days and the number of infections at 18 months. However, the children who showed the most behavioral distress (crying and longer times to quiet) to inoculation at 2 months had more infections at 18 months than those who were able to become quiet more quickly. The authors suggested that as an infant matures, it can better organize and control stressful situations. If there is an inability in an infant at 2 months of age to cope with stress the child may be more susceptible to illness.

Stress and Immunity: Sex Differences

Greenberg (1989) has stated that most females are immunologically superior to males. However, this immunological superiority, while an advantage in many diseases, can be disastrous in autoimmune diseases, where superior immune capabilities are brought to bear on the body's own

normal tissues. In terms of autoimmune disease, there is evidence for gender susceptibility with females being more prone to develop these diseases (Cohn, 1979; Greenberg, 1989; Michalski, Snyder, McLeod & Tahal, 1977; Soloman, 1981). The mechanisms underlying the gender difference are not known, but it is believed that depletion of T-suppressor cells causes autoimmune diseases by allowing unregulated T-helper cell function (Jackson, 1987).

Greenberg (1989) has stated the female sex hormone estrogen appears to be responsible for an enhanced immune response and that both humoral and cell-mediated systems appear to benefit from physiological amounts of estrogen. However, females afflicted with systemic lupus erythematosus (SLE) worsen after taking oral contraceptives that contain estrogen. If the ovaries are removed, the disease process is calmed, as the body no longer produces estrogen. Testosterone, a male sex hormone, stimulates suppressor T-lymphocytes which decreases humoral and cell mediated immune responses. Several researchers have suggested sex hormones are contributing factors in various autoimmune disease (Batchelor & Chapman, 1965; Dwyer, 1988; Geschwin & Behan, 1982), but others have suggested personality factors, such as masochism, self-sacrifice, denial of hostility, compliance-subservience, depression, and sensitivity to anger, may be related to the autoimmune disease rheumatoid arthritis (Soloman & Moos, 1964; Soloman, 1981).

Animal Studies

According to Greenberg (1989) experiments have shown both castrated males and normal females in certain strains of mice have shown higher concentrations of antibodies in response to antigens than did normal males. In addition an inbred strain of mouse, known as the New Zealand black hybrid, has been found to produce a disease much like SLE (Grade & Zegans, 1986). The female mouse develops the disease earlier in a more severe form than does the male. According to Rabin, Cunnick and Lysle (1988), if a male is castrated, it develops the disease as if it were female. The drug dihydrotestosterone has been found to decrease mortality in castrated females. Researchers have suggested that male androgen hormones promote the function of suppressor cells while the female sex hormone estrogen favors the development of helper T-cells. This section will present evidence concerning the importance of sex differences in host resistance, and the extent to which these differences may be influenced by stress.

Human Studies

In a nonclinical study, Thomas, Goodwin, and Goodwin (1985) examined the sex differences by looking at the effect of social support on stress-related changes in cholesterol, uric acid levels and immune function. The sample consisted of 256 males and females between the ages of 61 and 89 years. Immune function assessed included a total lymphocyte count and measurement of lymphocyte responses to the mitogen PHA. The Interview

Schedule for Social Interaction was employed to measure the presence of social bonds. Emotional status was measured with a self-rating checklist for symptoms of distress. Women reported a higher level of social support. The researchers reported that women overall had lower cholesterol and uric acid levels and better immune function. They postulated that social support may act to reduce the physiologic response to stress. With regard to the differences in the physiologic variables between men and women, they suggested that a greater sensitivity to close relationships and more versatility in their choice of relationships may help to buffer everyday stress in women.

Finally, in summary, according to Rabin, Cunnick, and Lysle (1988), sex differences in susceptibility to infection and disease may reflect differential responsiveness of the immune system. Jackson (1987) states it is believed that depletion of T-suppressor cells cause autoimmune disease by allowing unregulated T-helper-cell function. However, other researchers previously cited have suggested psychological factors may be the link between autoimmune diseases and stress.

Rationale and Hypotheses

The purpose of this study was to investigate the influence of early life experience (differential housing) on measures of immunocompetence (antibody formation, relative spleen weight, and T-cell counts) and neuroendocrine markers (glucose) associated with an immune response in male and female SD rats. More specifically, there appears to be little information concerning the

role of gender and the possible influence of an early life stressor on adult immunocompetence.

Because animal models provide the greatest control over extraneous variables, SD rats were the subjects in this investigation. In addition, the study employed an immunological challenge (SRBC) which did not kill the subjects, but was potent enough to elicit an antibody response. This antigen did not traumatize the animal unnecessarily (pain and suffering) like other agents (malaria and tuberculosis). More specifically, the antigen SRBC was selected as a challenge because antibody production to sheep erythrocytes has been reported as requiring T-lymphocyte interaction with antibody-producing B-lymphocytes before antibodies to SRBC can be produced. Thus, previous researchers have suggested that immunization with SRBC may be the most refined approach to use (Rabin, Cunnick, & Lysle, 1988).

Finally, there are limited data on the interaction between actual immunological challenge and endocrine functioning. In this study, glucose levels were obtained in an effort to assess the presence of stress. Asterita (1985) has stated that stress results in an elevated secretion of glucagon. Because glucagon converts stored glycogen to glucose, the level of glucose in the blood under stress should be elevated. Animal studies (Surwit & Feinglos, 1988) have demonstrated that 1 hour of standardized restraint and shaking produced significant increases in blood glucose in the ob/ob mouse. The same researchers found that stress was associated with a significant decrease in insulin in the ob/ob mouse but not in their lean littermates. In addition,

Huang, Plaut, Taylor, and Wareheim (1981) reported that blood glucose levels lessened over a period of 22 weeks in CD-1 male mice which had been injected with streptozotocin (STZ) and given light-shock stimulation. Thus, measures of glucose can also be used as an indicator of stress in both acute and chronic stress.

The hypotheses of this study are as follows: (1) individually housed animals will show more immunosuppression (decreased antibody titers, T-cells, spleen weight) as compared to group-housed animals; (2) individually housed females will show greater immunosuppression than will individually housed males; (3) individually housed animals will show higher glucose levels than will group housed animals; (4) individually housed animals will have lower body weights as compared to group housed animals; and (5) animals housed 3/cage will show more distress than those 6/cage as evidenced by greater immunosuppression.

CHAPTER II

MATERIALS AND METHODS

Subject Data

Animals and Housing

A total of 18 male and 18 female young (44 days old) Sprague-Dawley rats were employed in this study. Upon arrival from Harlan Laboratories (at approximately 30 days of age), the animals were allowed to adjust to vivarium conditions for 14 days. During this time, animals were group-housed (2/cage), according to AAALAC-approved colony housing. Purina Rat Chow and water were available ad lib. The animals were weighed weekly, and all animals were bled from the tail (via catheter) for glucose readings 14 days after arrival. Animals were placed on a 12:12 light-dark cycle, and room temperature was maintained at 21° C. After the 14 day adjustment period, all animals were weighed, identified via indelible ink markings on the tails, and then randomly assigned to one of three housing conditions: (1) singly housed, (2) 3/cage, and (3) 6/cage. Animals were segregated by sex and housed in 48 x 25 cm. polypropelene cages with 1225 cm^2 floor space available/animal. The animals remained in these housing conditions until the day of sacrifice, 36-38 days.

Immunological Challenge

Because of the immunological assessments employed in this study, the onset of treatment was staggered so that all groups of animals were immunized and sacrificed within 3 days of each other. Twenty-five ml of Sheep Red Blood Cells (SRBC) was obtained from U.T. College of Veterinary Medicine and stored in Alsever's solution. These cells were washed 3 times in phosphate-buffered saline (pH 7.1) and resuspended in saline to make a 10% suspension of SRBC. All rats were immunized (1 ml, i.p.) at the end of the housing manipulation (approximately 36-38 days) at approximately 80-82 days of age.

Harvesting Organs

Animals were sacrificed approximately 7 days after immunization with SRBC via decapitation. After initial injection, a latent period of approximately 1-2 weeks is required to elapse before antibody is detectable in the serum (Schwartz, 1982). In order to obtain optimum antibody formation of the primary antibody, IgG, the rats were sacrificed approximately 7 days post-immunization. Other researchers who have used SRBC as the antigen have sacrificed mice 4 days post-immunization (Michaut, Dechambre, Doumero, Lesourd, Devillechabrolle, & Moulias, 1981; Esterling & Rabin, 1987). Trunk blood was collected for the antibody-antigen agglutination assays and blood glucose determinations (Digital Glucometer). The spleens and thymi were removed by sterile techniques, and the spleens were weighed (S. Electronic

Balance). The thymi were placed in cold Hank's Balanced Salt Solution (HBSS), punctured with a 22 gauge needle and squeezed with forceps to obtain cells for counting. Ten ml of the thymus cell suspension was withdrawn and placed in a test tube on ice. After 5 minutes, it was transferred to another cold test tube and centrifuged for 10 minutes at 400 x g. The supernatant was discarded and the pellet resuspended in HBSS and centrifuged as before. After the supernatant was discarded, the pellet was resuspended in 1 ml of cold HBBS. The cells were then counted using a hemacytometer (Baxter Scientific Products).

Serology

Trunk blood was collected at the time of sacrifice and placed in centrifuge tubes. The blood was allowed to clot overnight at 4° C. The blood was then centrifuged at 400 x g (15 min.), and the serum drawn off, placed in microcentrifuge tubes, and stored (-20° C) for later analysis.

Titration

The antibody titer was determined by an agglutination assay using prepared immune serum and the antigen as follows:

(1) A 1:10 dilution of the control serum and antiserum (immunized serum) from each animal was prepared by adding 0.25 cc serum to 1 ml of NaCl solution.

(2) One row of 10 test tubes was set up for each animal.

(3) With a pipette, 0.25 cc NaCl solution was measured into each of the 10 tubes.

(4) To the first tube, 0.25 cc of the prepared antiserum dilution solution was added and mixed.

(5) Next 0.25 cc of this mixture was withdrawn and added to the second tube and mixed well. Then 0.25 cc of this mixture was transferred to the third tube, mixed and the procedure continued to tube #9.

(6) Tube #9 had 0.25 cc mixture withdrawn and discarded. Tube #10 contained only 0.25 cc NaCl solution without serum.

Thus the following serum dilutions were:

1. 1/20, 2. 1/40, 3. 1/80, 4. 1/160, 5. 1/320,

6. 1/640, 7. 1/1280, 8. 1/2560, 9. 1/5120, 10. Control

(7) Each tube had 0.25 cc of the 2% antigen, SRBC added with a 5 cc pipette. The final serum dilutions were:

1. 1/40, 2. 1/80, 3. 1/160, 4. 1/320, 5. 1/640

6. 1/1280, 7. 1/2560, 8. 1/5120, 9. 1/10240, 10. Control

(8) The dilution tubes were mixed well by shaking and transferred to the 37 degree incubator for 2 hours and then read.

Scoring Procedure

Direct agglutination results from the addition of antibody to a cell or to insoluble particulate native antigen. If the antigen is added to increasing dilutions of antiserum in tubes or wells with rounded bottoms, the reciprocal of

the dilution of antiserum in the last tube to show visible agglutination is the titer (relative concentration) of antibody (Jackson, 1987). Because of some imprecision, results in titration of 2 different specimens were considered significant only if end points differed by more than 2 dilution points.

Experimental Design

This study consisted of a 2 X 3 factorial design. Variable A was the sex of the animal (M and F) and variable B was differential housing conditions (1, 3, or 6 animals/cage). All dependent measures (antibody titers, glucose levels, spleen wt, thymus cell counts, and body weight) were analyzed using either a two-way ANOVA or repeated measures. Any significant F was further analyzed using the post hoc Scheffe's test. The design is depicted below:

	HOUSING		
SEX	1	3	6
Male	N=6	N=6	N=6
Female	N=6	N=6	N=6

CHAPTER III

RESULTS

Subject Data

Antibody Titers

The antibody titer was determined as the reciprocal of the highest dilution showing hemagglutination. The number of the titer (expressed in log units) reflects the number of antibody-producing plasma B cells. As predicted, there was a significant main effect of housing with regard to antibody formation [Appendix A, Table 8, $F(2,25) = 6.98$, $p < .05$]. Figure 1 illustrates the dependence of antibody titer data collapsed across gender on housing conditions. The Scheffe's analysis (Appendix A, Table 9) indicated that animals housed 1 to a cage showed a marked decrease in antibody titers compared with animals housed 6 to a cage. No other significant difference between the males and females were found. There was no significant difference between the males and females on antibody formation [Appendix A, Table 8, $F(1,25) = 0.4597$, $p > 0.05$].

T-Cell Enumeration

T-cell enumeration was determined by counting the total number of T-cells obtained from the thymus suspension and dividing by 2. Then this number was multiplied by .125 to obtain the number of cells per millimeter of

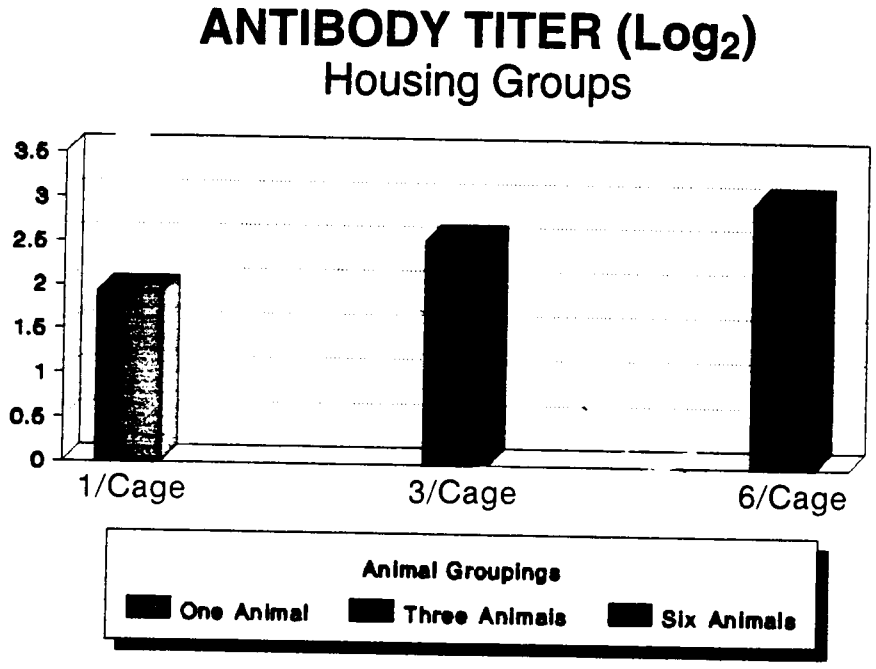

Figure 1. Antibody titers (log) collapsed across gender vs housing conditions.

suspension (expressed 10^6). No significant differences were found between the housing [Appendix A, Table 8, $F(2,30) = 1.25$, $p > .05$], or sex conditions [Appendix A, Table 8, $F(1,30) = 1.26$, $p > .05$] with regard to T-cell counts.

Glucose

Analysis of the housing manipulation on blood glucose responses yielded no significant differences [Appendix A, Table 8, $F(2,30) = 0.99$, $p > .05$]. However, there was a significant main effect of time [Appendix A, Table 8, $F(1,30) = 14.16$, $p < .05$]. The analysis indicated an overall reduction in blood glucose between the initial and final sample reads. In Figure 2 the concentration in blood glucose in mg/dl in males and females is plotted with respect to time. In addition, a significant interaction for time and sex [Appendix A, Table 8, $F(1,30) = 8.47$, $p < .05$] is shown in Figure 3. Post hoc analysis indicated that the males displayed a greater reduction in blood glucose concentration over time compared with the females.

Body and Organ Weights

Relative spleen weights in percent of body weight were calculated by dividing the actual spleen weight at sacrifice by the final body weight and multiplying by 100. A two-way ANOVA yielded no significant main effect of housing [Appendix A, Table 8, $F(2,30) = 0.45$, $p > .05$], but a significant main effect for sex was obtained [Appendix A, Table 8, $F(1,30) = 23.79$, $p < .05$]. When the data were collapsed across housing conditions, males

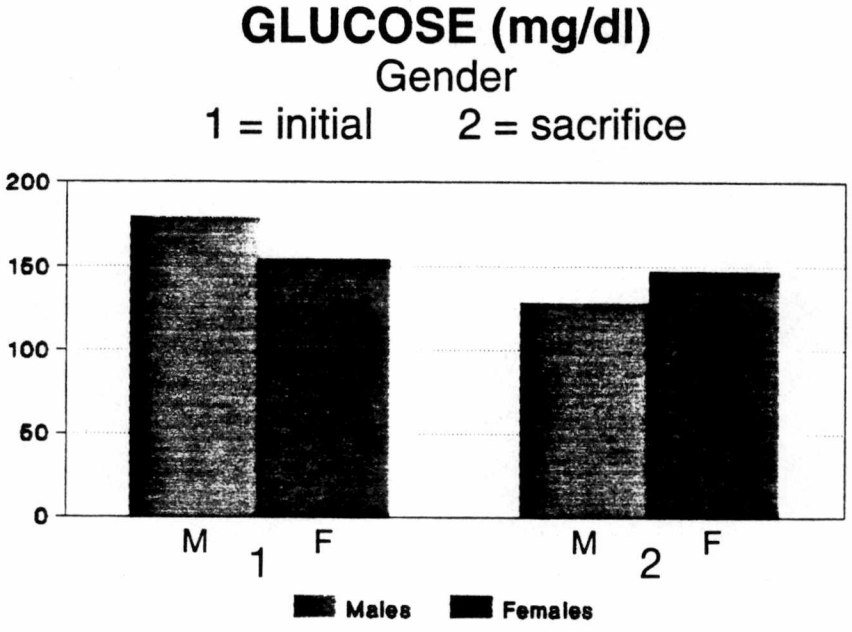

Figure 2. Mean blood glucose levels (mg/dl) collapsed across housing conditions with respect to time.

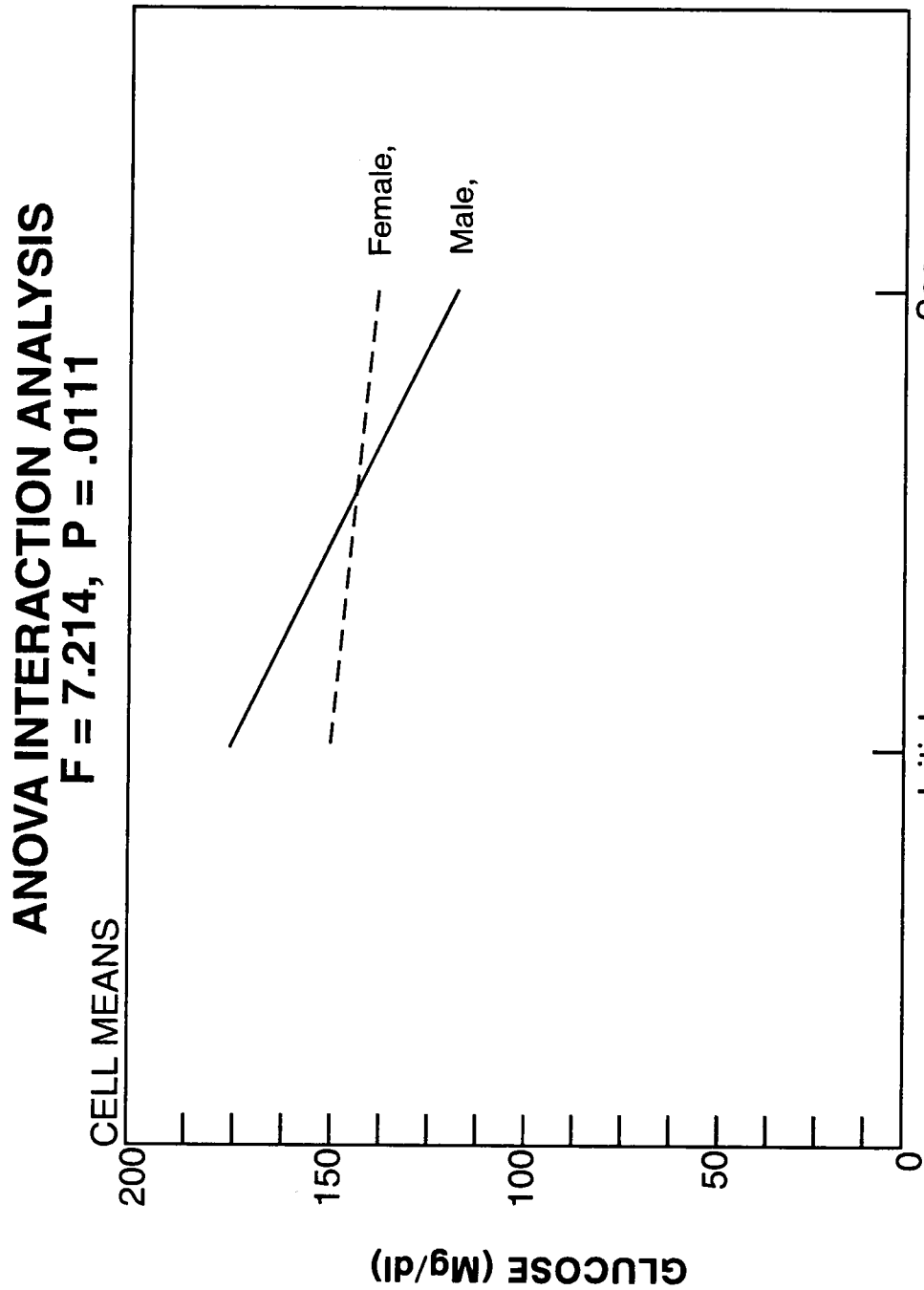

Figure 3. ANOVA interaction analysis of blood glucose with respect to sex.

displayed smaller relative spleen weights than the female (see Figure 4).

The hypothesis that individually housed animals would show lower body weights than group housed animals at sacrifice was not supported. However, there were significant differences among the sexes [$F(1,30) = 318.78$, $p < .05$] (Appendix A; Figure 5). The males weighed more both initially and throughout the study. However, there was no significant interaction between the housing and sex conditions [$F(2,30) = 0.30$, $p > .05$]. Figure 5 represents weekly body weight differences with regard to sex.

Dependent Measures Correlation Matrix

In order to assess whether or not there was an association between the immune and endocrine measures, several Pearson product moment correlations were computed. The analyses, presented in Tables 1-7, show a significantly positive relationship between relative spleen weight and antibody responsiveness to the antigen SRBC with regard to the female subjects (see Table 1). However, there was a significantly negative relationship between antibody responsiveness to SRBC and blood glucose concentration in the male subjects (see Table 2). According to Kelley, Mahoney, Randich, and West (1990) the stress response itself has been found to be a function of sex as the resting levels of corticosterone are higher than that of males and the corticosteroid response to stress is greater in magnitude and duration in female rats than in males. The differences found between the sexes may be related to these reported corticosteroid responses to stress.

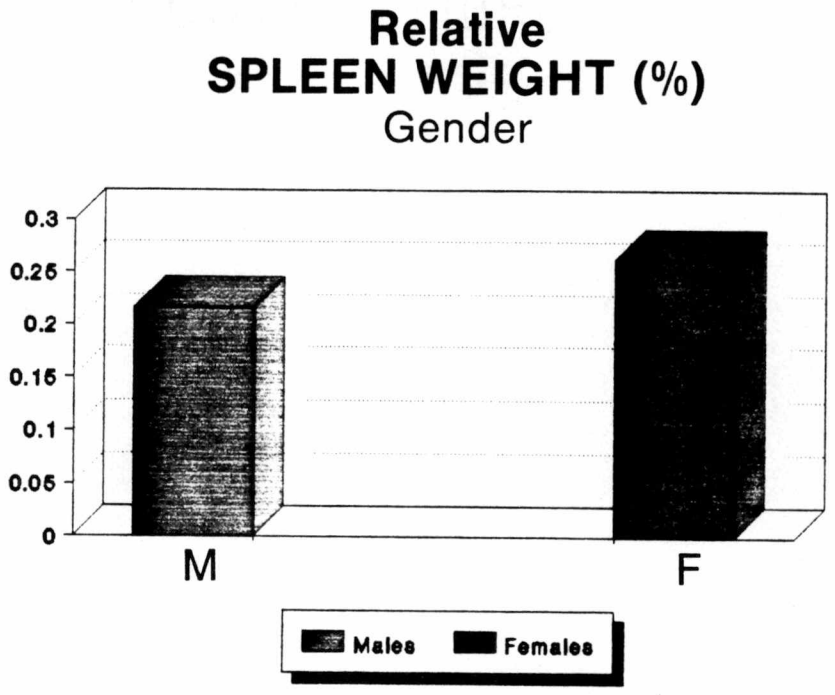

Figure 4. Relative spleen weight (%) collapsed across housing conditions.

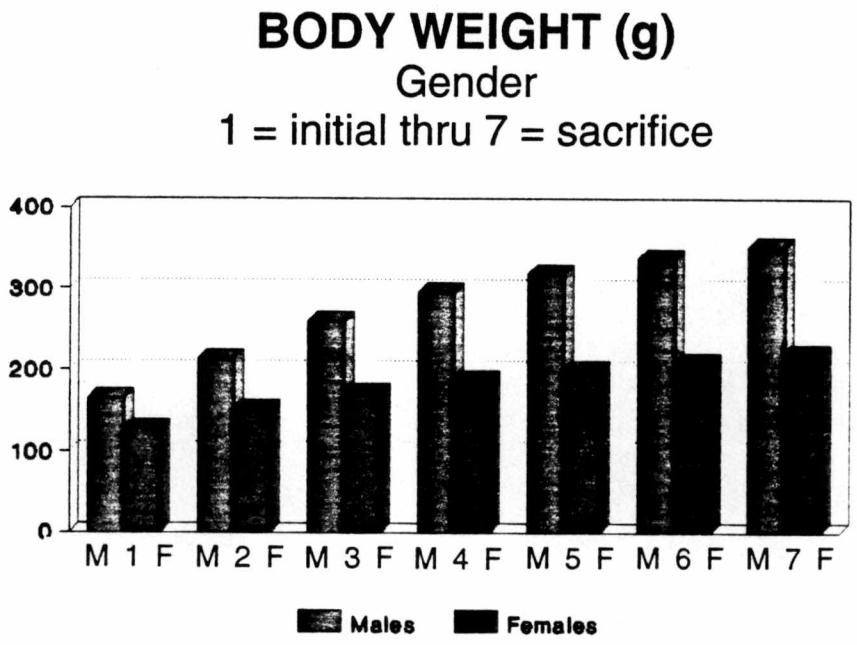

Figure 5. Mean body weight (grams) per week collapsed across housing conditions.

Table 1. Correlation matrix--females: all housing conditions, relative spleen weight vs. titer

	1	2
Subject data	Relative spleen weight	Titer
Relative spleen weight (N = 18)	1	.5739*
Titer (N = 16)	.5739*	1

*p < .05

Table 2. Correlation matrix--males: all housing conditions, titer vs. glucose

	1	2
Subject data	Titer	Glucose
Titer (N = 15)	1	-.58231*
Glucose (N = 18)	-.58231*	1

*p < .05

Table 3. Correlation matrix--males: all housing conditions, relative spleen weight vs. titer

	1	2
Subject data	Relative spleen weight	Titer
Relative spleen weight (N = 18)	1	-.04257
Titer (N = 15)	-.04257	1

*p < .05

Table 4. Correlation matrix--females: all housing conditions, titer vs. glucose

	1	2
Subject data	Titer	Glucose
Titer (N = 16)	1	.07883
Glucose (N = 18)	.07883	1

*p < .05

Table 5. Correlation matrix--males and females 1/cage, titer vs. glucose

	1	2
Subject data	Titer	Glucose
Titer (N = 12)	1	.12451
Glucose (N = 12)	.12451	1

*p < .05

Table 6. Correlation matrix--males and females 3/cage, titer vs. glucose

	1	2
Subject data	Titer	Glucose
Titer (N = 9)	1	-.48676
Glucose (N = 12)	-.48676	1

*p < .05

Table 7 Correlation matrix--males and females 6/cage, titer vs. glucose

	1	2
Subject data	Titer	Glucose
Titer (N = 10)	1	.37147
Glucose (N = 12)	.37147	1

*p < .05

CHAPTER IV

DISCUSSION

Generally, there has been agreement by researchers that differential housing is a stressor that produces a number of behavioral and physiological changes in rodents. However, little attention has been given to the issue of exposing organisms early in life to this stressor and measuring the effect on adult immunocompetence. In addition, there are limited studies regarding the effects of stressors on various female physiological outcome (Cotton, 1990).

The purpose of this study was to investigate the effects of early exposure to differential housing on adult immunocompetence and to examine any sex differences in various immune and endocrine measures with regard to this type of stressor.

In terms of antibody formation, it was hypothesized that rats housed individually would show lower antibody titers than would group-housed animals. In addition, it was hypothesized that individually-housed females would show a lower antibody formation response compared with individually-housed males. The first hypothesis was supported, in that individually-housed rats were found to show lower titers than did group-housed animals, but no differences were found between males and females.

According to O'Leary (1990) the level of specific antibodies is thought to reflect the competence of the T cells in keeping the virus

sequestered and latent. Thus, for clinical purposes, the higher the antibody titer, the greater the immune response, the greater the infection. However, lower antibody titers could be caused by any of several mechanisms. First, impaired immunity could be due to stressor interfering with T-cell regulation. An imbalance in the ratio of the regulatory T-helper to T-suppressor cells and/or macrophages may result from infection, stress, and malignancy (Jackson, 1987). Taylor and Ross (1989) have suggested that stress may alter antibody response by direct action on B cells or indirectly by preventing the activation and/or functional activity of regulatory T lymphocytes. Secondly, stressor influence on the clearance rate (from blood to tissue) of an antigen producing smaller immune response. In other words, the rapid elimination of the antigen from the serum will produce a lower antibody titer when tested. It is known that the kinetics of clearance of labeled antigen retained in the injected leg tissue is stable and remains so for many weeks (Tew & Mandel, 1978). Finally, lower antibody titers could be due to increased corticosterone activity due to stress (Riley, Fitzmaurice & Spackman, 1981). Suter (1986) has suggested that although cellular immunity is primarily impaired by stress via corticosteroid release, humoral immunity is also impaired, possibly because T-cells regulate the function of B-cells that are essential in the formation of antibodies.

The primary finding of suppressed antibody formation with isolation is consistent with an earlier study by Glenn and Becker (1969) who reported that mice housed individually showed lower antibody titers than did animals housed

in groups of 6. These researchers demonstrated for the first time that immunization with a foreign protein involved an immunological response that could be affected by the psychological experience of separation from other mice. Riley, Fitzmaurice, and Spackman (1981) have also suggested that this study indicated that the immune capabilities of the mice housed singly were impaired in comparison with those housed in a more "normal" crowded social situation.

However, several other studies have reported that crowding, rather than isolation, produces lower antibody titers in animals. Vessey (1964) housed C3H male mice of 9 to 15 weeks of age in groups of 5-6/cage or 1/cage. On the 5th day of manipulation, the mice were injected with the antigen, beef serum. The researcher reported antibody titers generally increased by the 11th day followed by a decline for all mice. However, mean titers of the isolated mice were found to be significantly higher than those of the grouped animals for days 11 and 18. In addition, it was noted that social rank appeared to affect antibody formation in that dominant mice were found to show significantly higher titers than the more submissive mice. The researchers suggested that the stress of crowding and submissiveness may have increased the output of adrenocortical hormones resulting in inhibition of antibody formation.

Edwards, Rahe, Stephens, and Henry (1980) reared CBA/USC male mice in thee different housing groups to investigate the effects of psychosoocial environmental change on antibody response to a challenge of

bovine serum albumin. Control animals were raised in isolation throughout the experiment, the second group was moved from isolation to a population cage at 16 weeks of age, and a third group was first isolated, then grouped, and returned to isolation. At 16 weeks of age all mice were injected with the antigen. The researchers reported that mice in continuous isolation had significantly higher antibody levels than mice in the other two groups. Mice that were initially isolated, then grouped, and later re-isolated had the lowest antibody levels. The conclusion drawn was that psychosocial environmental changes can significantly suppress antibody formation in mice.

Brain and Benton (1979) have suggested that species differences in the social organization may influence results of isolation versus grouping comparisons. For example, in the wild, rats live in large groups while mice are solitary. The present finding that individually-housed rats showed a lower antibody response than did groups of either 3 or 6/cage appears to lend support to this hypothesis. Based upon this proposition, one may infer isolation to have a more detrimental effect on rats than on mice. Therefore, the findings of this study suggest rats housed individually were more affected by their unusual housing conditions than individually-housed mice.

However, this argument is not consistent with the findings of Glenn and Becker (1969) who reported mice housed singly were immunologically impaired in comparison with crowded mice. In addition, other studies do not support this interpretation (Dechambre & Gosse, 1973; Cooley, Henry, &

Stephens, 1976; Edwards & Dean, 1977; Jessop, Gale, & Bayer, 1987; Joasoo & McKenzie, 1976; Plaut, Ader, Friedman, & Ritterson, 1969).

Although some researchers have reported females to have the capacity to outperform males in terms of immune responsiveness (e.g., antibody production), the present study did not find a significant difference between male and female subjects with respect to this dependent measure. However, relative spleen weight was positively correlated with antibody formation in the female, while males displayed a significantly smaller relative spleen weight across the housing conditions. Previous researchers (Brain & Stanislaw, 1982; Hatch, Wiberg, Zawidzka & Cann, 1964) reported gender differences in adrenal weight collapsed across stress conditions. The finding from the present study lends support for possible gender differences among immune parameters. According to Elliot and Eisdorfer (1982), genetic factors may play a more pronounced role in immunoregulation than previously recognized. More specifically, they argue that the regulation of catecholamines may be genetically determined and thus produce genetic differences in responding to stressful situations.

It is well documented that exposure to stressful stimuli is accompanied by increased sympatho-adreno-medullary and pituitary-adrenocortical activity, which produces raised plasma catecholamines and glucocorticoid levels (Armario, Montero, & Balasch, 1985). Therefore, it was hypothesized that individually-housed rats would show higher blood glucose levels than those housed in groups. This hypothesis was not confirmed. In fact, the present

finding of overall reduction in glucose with time contradicts previous studies (Odio & Maickel, 1984; Surwit & Feinglos, 1988) that report increased levels of glucose with stress.

However, Huang, Plaut, Taylor, and Wareheim (1981) also reported a decrease in glucose levels over time. Young male CD-1 mice were stressed by periodic light-shocks in order to investigate the incidence of streptozotocin-induced (STZ) diabetes. Blood glucose levels were reported to lessen over a 22-week period in mice that were stimulated with light-shock during the first 72 hours after injection of STZ, while nonstimulated mice were found to have significantly higher glucose levels. The authors suggested corticosterone responses to the light-shock stress may have lessened the effect of STZ on the beta cells in islets and inhibited the development of insulin dependent diabetes.

Armario, Castellanos, and Balasch (1984) reported that chronic noise modified neither glucose nor insulin levels in 8 week old male Wistar rats which were exposed to 5 or 21 days of chronic noise stress. In addition, they reported that chronic noise did not modify the weight of the endocrine glands nor the basal levels of anterior pituitary hormones and corticosterone. The researchers concluded the data suggested that physiological factors controlling insulin secretion during acute and chronic stress are poorly understood and require further study.

Hennessey and Levine (1978) reported that adult male C57BL/KA mice which were gently picked up by their tails and returned to their home cages did

not differ in resting and after handling corticosterone concentrations. However, mice which had been exposed to differing environmental conditions were found to show significant increases in mean plasma corticosterone levels as conditions were increasingly dissimilar.

Therefore, although it is possible that the present findings regarding decreased glucose levels are confounded by maturation of the subjects or handling, these studies tend to support the findings that chronic stress in this study were not necessarily confounded by these factors.

De Boer, Koopmans, Slangen, and Van Der Gugten (1990) investigated the response of plasma catecholamine, corticosterone, and glucose to acute, repeated exposure to stressful stimuli. They reported that rats subjected to water immersion (WIS) initially showed marked increases in plasma noradrenaline, adrenalin, corticosterone, and glucose. However, the magnitude of these water immersion-induced responses gradually declined with repeated exposure. The researchers suggested that an attenuation of sympathetic-adrenomedullary and pituitary-adrenocortical responses during repeated exposure to the same stressor permitted conservation of energy by dampening mobilization of glucose.

Hatch, Wiberg, Cann, Airth, and Grice (1964) described an "isolation syndrome" in rats. They reported lower body, splenic and thymic weights in isolated as compared to community-housed (10/cage) rats. However, Plaut, Ader, Friedman, and Ritterson (1969) reported no significant differences in body weights of female Charles River CD 1 mice housed in 1, 5,

or 20/cage. Likewise, Beden and Stanislow (1982) investigated the effects of isolation on body weights in male and female mice. They reported no significant body weight differences between the housing conditions. Housing conditions did not appear to have an effect on body weight in either male or female animals in the present study.

Finally, the finding that males displayed a greater overall reduction in blood glucose levels which was inversely related to antibody formation is of interest. This finding seems to support the general belief that the underlying mechanism for immunosuppression is glucocorticoid related (Riley, Fitzmaurice, & Spackman, 1981), at least in males.

To summarize, the results were found to be mixed with regard to the immune parameters investigated. Individually housed animals were shown to have the least antibody response, while the groups of 6/cage showed the highest. Glenn and Becker (1969) have suggested that fewer antibodies observed in response to an antigen indicates that immune capabilities are suppressed. However, Cohen and Williamson (1991) have argued it is not clear that effects of stress on immune changes are immunosuppressive. They have suggested that even several measures of immune function may not be an adequate representation of host resistance (Palmbad, 1981; Plaut & Friedman, 1981). These findings also suggest that exposure to an early-life stressor may compromise adult immunocompetence (as indicated by the lower antibody titers observed in the individually-housed animals), regardless of sex.

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APPENDIXES

APPENDIX A

ANOVA SUMMARY TABLES

Table 8. Statistical Analyses

Source	Degrees of freedom	Type IIISS	Mean square	F value	Pr > P
Two-way ANOVA--Dependent variable = TITER					
Sex	1	0.2525	0.2525	0.56	0.4597
Treatment	2	6.2521	3.1261	6.98	<.05
Sex*TRT	2	1.7629	0.8815	1.97	0.1607
Two-way ANOVA--Dependent variable = Relative spleen weight (%)					
Sex	1	0.0205	0.0205	23.79	<.05
Treatment	2	0.0007	0.0003	0.45	0.6422
Sex*TRT	2	0.0030	0.0015	1.79	0.1844
Two-way ANOVA--Dependent variable = TCELLS					
Sex	1	1782.23	1782.23	1.26	0.2701
Treatment	2	3514.50	1757.25	1.25	0.3026
Sex*TRT	2	0068.18	0034.09	0.02	0.9761
Repeated measures ANOVA--Dependent variable = Body weight					
Sex	1	587791.68	587791.68	318.78	<.05
Treatment	2	419.90	209.95	0.11	0.8928
Sex*TRT	2	1107.14	553.57	0.30	0.7429
Repeated measures ANOVA--Dependent variable = Glucose (mg/dl)					
Sex (B)	1	200.00	200.00	0.1589	.6927
Time (C)	1	15143.83	15143.83	18.136	<.05
Housing (A)	2	607.43	303.71	0.3023	.7413
A*B	1	9068.34	9068.34	7.2140	.0111
A*C	2	2061.50	1030.75	.9638	.3929
B*C	1	9067.51	9067.51	8.4788	<.05
A*B*C	2	5443.91	2721.96	2.5452	.0953

Table 9. Scheffe's Test for Titer (TRT)

Housing TRT Comparison[a]		Simultaneous Lowest Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
6/cage 3	-2	-0.3518	0.4482	1.2481
3	-1	0.3131	1.0586	1.8041***[b]
3/cage 2	-3	-1.2481	-0.4482	0.3518
2	-1	-0.1573	0.6104	1.3782
1/cage 1	-3	-1.8041	-1.0586	-0.3131***[b]
1	-2	-1.3782	-0.6104	0.1573

[a]For this test Alpha = 0.05, Confidence = 0.95, DF = 25, MSE = 0.447731, Critical value for F = 3.38519.

[b]Comparisons significant at the 0.05 level are indicated by ***.

APPENDIX B

SUBJECT DATA

The experimental data are presented in Tables 10-21. The average values and their uncertainties of the different groupings of subjects are given in Tables 22-32. The uncertainties given in Tables 22-32 are the standard deviations of the mean.

Table 10. Glucose, Spleen Weight, and Lymphocytes for Individually-Housed Male Rats

	Glucose (mg/dl)		Spleen		Lymphocytes	
Subject number	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
6	126.5	137.0	0.849	0.2643	1.602	48.4
5	153.0	135.0	0.660	0.2061	1.301	19.5
3	139.5	150.0	0.781	0.2241	1.602	101.7
4	172.0	160.0	0.841	0.2283	2.505	--
14	175.0	134.0	0.808	0.2150	1.903	51.6
13	155.0	121.0	0.774	0.2110	2.505	175.4

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 11. Glucose, Spleen Weight, and Lymphocytes for Male Rats Housed in Groups of 3

Subject number	Glucose (mg/dl)		Spleen		Lymphocytes	
	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
8	193.5	136.0	0.791	0.2217	3.107	108.6
9	164.0	129.0	0.757	0.2008	--	150.4
10	215.5	149.0	0.822	0.2208	1.903	113.8
7	210.0	122.0	0.601	0.1840	--	55.6
12	146.0	125.0	0.596	0.1850	2.505	85.0
11	175.5	89.5	0.880	0.2172	3.408	86.5

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 12. Glucose, Spleen Weight, and Lymphocytes for Male Rats Housed in Groups of 6

Subject number	Glucose (mg/dl)		Spleen		Lymphocytes	
	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
17	219.6	142.0	0.737	0.2181	1.602	160.2
18	229.0	125.0	0.818	0.2381	3.107	101.0
1	187.0	106.0	0.752	0.2195	2.806	38.8
2	280.0	113.0	0.755	0.2317	2.806	93.3
15	147.5	124.0	0.634	0.1819	--	78.1
16	151.0	116.0	0.846	0.2243	2.505	42.1

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 13. Glucose, Spleen Weight, and Lymphocytes for Individually-Housed Female Rats

	Glucose (mg/dl)		Spleen		Lymphocytes	
Subject number	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
19	285.5	156.0	0.546	0.2739	3.709	29.0
20	132.5	96.0	0.565	0.2660	1.903	75.7
25	131.0	157.0	0.462	0.1975	1.602	54.0
26	159.0	126.0	0.627	0.2782	1.301	125.2
21	100.5	175.0	0.463	0.2107	1.301	40.4
22	148.5	148.0	0.640	0.2561	1.903	47.9

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 14. Glucose, Spleen Weight, and Lymphocytes for Female Rats Housed in Groups of 3

Subject number	Glucose (mg/dl)		Spleen		Lymphocytes	
	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
31	121.0	146.0	0.616	0.2987	3.408	94.1
32	195.0	183.0	0.522	0.2168	1.903	81.4
29	136.0	166.0	0.549	0.2609	--	104.8
23	178.6	129.0	0.575	0.2797	3.107	87.2
24	160.0	133.0	0.540	0.2423	1.301	46.3
30	127.0	145.0	0.745	0.3255	2.204	122.9

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 15. Glucose, Spleen Weight, and Lymphocytes for Female Rats Housed in Groups of 6

Subject number	Glucose (mg/dl)		Spleen		Lymphocytes	
	Initial	Final	Weight grams	Relative weight (%)[a]	Log B cells	T cell cell/ml
27	202.0	137.0	0.648	0.2794	3.408	60.2
28	155.5	151.0	0.663	0.2655	--	45.1
35	121.0	148.0	0.465	0.2626	3.107	89.9
36	133.0	156.0	0.700	0.3273	3.408	65.9
33	184.6	120.0	0.688	0.3431	3.408	78.2
34	109.5	185.5	0.554	0.2574	3.709	83.8

[a]Relative spleen weight (%) is the spleen weight divided by the total body weight multiplied by 100.

Table 16. Body Weights for Individually Housed Male Rats

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
6	148.8	191.0	230.7	268.2	287.8	306.9	321.2
5	157.7	201.2	243.7	274.2	293.0	307.5	320.3
3	145.1	191.1	244.8	282.5	305.1	332.5	348.5
4	166.1	224.1	271.5	308.8	330.8	354.8	368.4
14	178.4	235.2	281.5	322.4	341.3	364.7	375.8
13	150.1	206.2	253.7	307.6	330.2	353.3	365.1

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 17. Body Weights for Individually Housed Female Rats

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
19	119.0	144.4	157.5	174.6	181.5	193.9	199.3
20	112.3	134.2	155.9	175.8	188.1	202.6	212.4
25	149.3	166.1	187.1	198.2	208.5	220.8	233.9
26	117.1	143.5	168.2	192.1	200.3	214.8	225.4
21	125.3	149.2	170.5	192.0	194.4	208.4	219.7
22	137.1	167.5	191.5	208.9	225.8	241.6	249.9

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 18. Body Weights for Male Rats Housed in Groups of 3

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
8	152.9	207.9	256.4	299.8	327.2	339.4	356.8
9	157.3	211.7	260.8	305.1	326.9	352.6	376.9
10	155.5	210.8	260.9	302.1	335.4	358.4	372.3
7	169.4	222.5	262.8	292.3	305.7	313.5	326.7
12	146.7	191.5	234.0	268.7	293.7	307.8	322.1
11	184.1	248.3	299.2	341.8	370.2	381.2	405.1

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 19. Body Weights for Female Rats Housed in Groups of 3

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
31	121.0	142.6	162.6	176.6	192.8	201.4	206.2
32	138.2	166.0	186.6	203.5	218.0	229.0	240.8
29	127.0	152.3	166.6	181.8	196.6	203.9	210.4
23	134.2	158.4	180.4	173.8	187.9	198.1	205.6
24	121.8	151.3	166.5	183.8	195.8	213.3	222.9
30	110.5	140.5	158.5	192.2	205.1	204.6	228.9

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 20. Body Weights for Male Rats Housed in Groups of 6

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
17	189.8	206.4	251.3	280.1	306.6	326.3	337.9
18	210.9	230.4	280.6	292.6	341.2	359.2	343.6
1	168.0	219.5	265.8	299.1	317.7	334.2	342.6
2	154.8	204.8	251.3	278.2	298.7	314.6	325.8
15	169.1	223.6	251.9	313.0	318.3	337.7	348.6
16	155.7	206.9	267.6	284.2	312.7	336.7	377.2

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 21. Body Weights for Female Rats Housed in Groups of 6

Subject number	Body weight[a] (gms)						
	4-3 ini	4-11	4-18	4-25	5-2	5-9	5-13 5-15 sac
27	125.3	146.3	167.6	189.2	202.2	207.7	231.9
28	150.1	176.1	198.3	221.5	240.1	247.3	249.7
35	122.3	137.8	156.2	172.8	180.3	196.6	177.1
36	118.7	140.9	157.7	159.9	170.2	170.3	213.9
33	141.7	164.0	178.0	191.8	198.8	192.1	200.5
34	118.5	145.9	169.9	182.1	197.7	208.9	215.2

[a]ini signifies initial weight and sac signifies weight at sacrifice.

Table 22. Average Values and Standard Deviations for Individually Housed Male Rats

Variables[a]	Average values
Body weight (gms)	
Initial	157.7 ± 5.1
Week 1	208.1 ± 7.3
Week 2	254.3 ± 7.7
Week 3	294.0 ± 8.9
Week 4	314.7 ± 9.1
Week 5	336.6 ± 10.2
Sacrifice	348.1 ± 9.5
Glucose (mg/dl)	
Initial	153.5 ± 7.6
Sacrifice	139.5 ± 5.5
Relative spleen weight (%)	0.2248 ± 0.0086
Log B cells	1.903 ± 0.206
T cell count (cells/ml)	79.3 ± 27.4

[a] The value of N was 6 for all variables except the T cell count where N was 5.

Table 23. Average Values and Standard Deviations for Male Rats Housed in Groups of 3

Variables[a]	Average values
Body weight (gms)	
Initial	160.9 ± 5.6
Week 1	215.5 ± 7.7
Week 2	262.4 ± 8.5
Week 3	301.6 ± 9.7
Week 4	326.5 ± 10.7
Week 5	342.2 ± 11.4
Sacrifice	339.9 ± 12.9
Glucose (mg/dl)	
Initial	184.0 ± 11.1
Sacrifice	125.0 ± 8.1
Relative spleen weight (%)	0.2049 ± 0.0071
Log B cells	2.730 ± 0.333
T cell count (cells/ml)	99.9 ± 16.1

[a] The value of N was 6 for all variables except the antibody titer where N was 4.

Table 24. Average Values and Standard Deviations for Male Rats Housed in Groups of 6

Variables[a]	Average values
Body weight (gms)	
Initial	174.7 ± 8.9
Week 1	213.3 ± 4.4
Week 2	261.4 ± 4.9
Week 3	291.2 ± 5.4
Week 4	313.9 ± 5.9
Week 5	334.8 ± 6.0
Sacrifice	345.9 ± 7.0
Glucose (mg/dl)	
Initial	202.4 ± 20.7
Sacrifice	121.0 ± 5.1
Relative spleen weight (%)	0.2189 ± 0.0080
Log B cells	2.565 ± 0.259
T cell count (cells/ml)	83.6 ± 18.2

[a] The value of N was 6 for all variables except the antibody titer where N was 5.

Table 25. Average Values and Standard Deviations for Individually Housed Female Rats

Variables[a]	Average values
Body weight (gms)	
Initial	126.7 ± 5.7
Week 1	150.8 ± 5.4
Week 2	171.8 ± 6.0
Week 3	190.3 ± 5.4
Week 4	199.8 ± 6.5
Week 5	213.7 ± 6.8
Sacrifice	223.4 ± 7.1
Glucose (mg/dl)	
Initial	139.5 ± 26.5
Sacrifice	143.0 ± 11.4
Relative spleen weight (%)	0.2170 ± 0.0140
Log B cells	1.953 ± 0.367
T cell count (cells/ml)	62.0 ± 14.1

[a] The value of N was 6 for all variables.

Table 26. Average Values and Standard Deviations for Female Rats Housed in Groups of 3

Variables[a]	Average values
Body weight (gms)	
Initial	123.4 ± 4.0
Week 1	131.8 ± 3.9
Week 2	169.1 ± 4.0
Week 3	186.4 ± 4.0
Week 4	199.4 ± 4.4
Week 5	210.0 ± 4.7
Sacrifice	219.1 ± 5.8
Glucose (mg/dl)	
Initial	152.9 ± 12.2
Sacrifice	130.3 ± 8.8
Relative spleen weight (%)	0.2707 ± 0.0160
Log B cells	2.381 ± 0.387
T cell count (cells/ml)	89.4 ± 10.5

[a] The value of N was 6 for all variables except the antibody titer where N was 5.

Table 27. Average Values and Standard Deviations for Female Rats Housed in Groups of 6

Variables[a]	Average values
Body weight (gms)	
Initial	129.4 ± 5.4
Week 1	131.8 ± 6.1
Week 2	171.3 ± 6.3
Week 3	186.2 ± 8.5
Week 4	198.2 ± 9.8
Week 5	203.8 ± 10.4
Sacrifice	214.7 ± 10.2
Glucose (mg/dl)	
Initial	150.9 ± 14.9
Sacrifice	149.6 ± 8.9
Relative spleen weight (%)	0.2742 ± 0.0148
Log B cells	3.408 ± 0.094
T cell count (cells/ml)	70.5 ± 6.8

[a] The value of N was 6 for all variables except the antibody titer where N was 5.

Table 28. Average Values and Standard Deviations for Male Rats (Includes All Housing Conditions)

Variables[a]	Average values
Body weight (gms)	
Initial	164.5 ± 4.1
Week 1	212.9 ± 3.7
Week 2	259.4 ± 4.0
Week 3	295.6 ± 4.6
Week 4	319.0 ± 5.0
Week 5	337.9 ± 5.2
Sacrifice	351.3 ± 5.7
Glucose (mg/dl)	
Initial	179.9 ± 9.1
Sacrifice	128.5 ± 4.0
Relative spleen weight (%)	0.2162 ± 0.0047
Log B cells	2.341 ± 10.7
T cell count (cells/ml)	88.8 ± 10.7

[a] The value of N was 18 for all variables except the antibody titer where N was 15 and T cell count where N was 17.

Table 29. Average Values and Standard Deviations for Female Rats (Includes All Housing Conditions)

Variables[a]	Average values
Body weight (gms)	
Initial	127.2 ± 2.8
Week 1	131.5 ± 2.8
Week 2	170.7 ± 3.0
Week 3	187.6 ± 3.4
Week 4	199.1 ± 3.9
Week 5	209.2 ± 4.3
Sacrifice	219.1 ± 4.4
Glucose (mg/dl)	
Initial	154.5 ± 10.3
Sacrifice	147.6 ± 5.3
Relative spleen weight (%)	0.2639 ± 0.0086
Log B cells	2.543 ± 0.235
T cell count (cells/ml)	73.9 ± 6.5

[a] The value of N was 18 for all variables except the antibody titer where N was 16.

Table 30. Average Values and Standard Deviations for Individually Housed Male and Female Rats

Variables[a]	Average values
Body weight (gms)	
Initial	142.2 ± 5.9
Week 1	179.5 ± 9.7
Week 2	213.1 ± 13.3
Week 3	242.1 ± 16.2
Week 4	257.2 ± 18.1
Week 5	275.1 ± 19.4
Sacrifice	285.7 ± 19.6
Glucose (mg/dl)	
Initial	156.6 ± 13.2
Sacrifice	141.3 ± 6.1
Relative spleen weight (%)	0.2359 ± 0.0085
Log B cells	1.928 ± 0.201
T cell count (cells/ml)	69.9 ± 14.1

[a] The value of N was 12 for all variables except the T cell count where N was 11.

Table 31. Average Values and Standard Deviations for Male and Female Rats Housed in Groups of 3

Variables[a]	Average values
Body weight (gms)	
Initial	143.2 ± 6.3
Week 1	183.7 ± 10.7
Week 2	215.7 ± 14.8
Week 3	244.0 ± 18.1
Week 4	262.9 ± 19.9
Week 5	276.1 ± 20.8
Sacrifice	289.6 ± 22.3
Glucose (mg/dl)	
Initial	168.5 ± 9.2
Sacrifice	137.7 ± 6.8
Relative spleen weight (%)	0.2377 ± 0.0130
Log B cells	2.538 ± 0.253
T cell count (cells/ml)	94.7 ± 8.2

[a] The value of N was 12 for all variables except the antibody titer where N was 9.

Table 32. Average Values and Standard Deviations for Male and Female Rats Housed in Groups of 6

Variables[a]	Average values
Body weight (gms)	
Initial	152.1 ± 29.2
Week 1	183.5 ± 35.4
Week 2	216.3 ± 48.9
Week 3	238.7 ± 57.3
Week 4	257.0 ± 64.3
Week 5	269.3 ± 71.2
Sacrifice	280.3 ± 71.5
Glucose (mg/dl)	
Initial	176.6 ± 50.0
Sacrifice	135.3 ± 22.6
Relative spleen weight (%)	0.2465 ± 0.0401
Log B cells	2.987 ± 0.605
T cell count (cells/ml)	78.0 ± 33.1

[a] The value of N was 12 for all variables except the antibody titer where N was 10.

VITA

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