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**The effect of age and gender on the peripheral blood cell
response to *Escherichia coli* lipopolysaccharide (LPS) in Wistar
rats (*Rattus norvegicus*)**

Merritt, Deborah Jean, Ph.D.

University of Hawaii, 1994

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THE EFFECT OF AGE AND GENDER ON THE PERIPHERAL BLOOD CELL
RESPONSE TO *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE (LPS) IN
WISTAR RATS (*Rattus Norvegicus*)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE
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DOCTOR OF PHILOSOPHY

IN

BIOMEDICAL SCIENCES

(PHYSIOLOGY)

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ABSTRACT

Escherichia coli (*E. coli*) lipopolysaccharide (LPS) is the major component of mammalian gut bacterial flora. Inflammatory diseases of unknown etiology, e.g., rheumatoid arthritis, may be triggered by periodic transmural release of LPS from the gut into the peritoneal cavity. If so, an older animal should have a greater exposure history to LPS and, therefore, be hyper-responsive.

Age and gender-related variation in peripheral blood cell responsiveness was determined in untreated rats (time zero). The acute (0-2 day) and long-term (3-24 day) response to an intraperitoneal injection of 0.5 mg/kg *E. coli* LPS was compared to control (sterile 0.9% saline) in young, middle-aged, and old male and female Wistar rats.

In untreated rats, a number of parameters changed with age: older rats had increased white blood cell (WBC) chemiluminescence (CL), time-to-peak CL, plasma protein concentration, and decreased WBC total count, and *in vitro* WBC mobility. Packed cell volume (PVC) increased in middle-age, but decreased in old rats.

Saline-treated male rats had higher WBC counts, body weight, CL, and PVC when compared to age-matched, saline-treated, female rats.

Acutely, LPS caused a hypothermia in all rats, which was more profound and prolonged in the old rats. Hypothermia

was followed by fever, which was highest in the young rats. LPS also increased WBC counts and CL, and decreased time-to-peak WBC CL in all rats.

WBC CL was greater in old and middle-aged rats during days 13-24 following LPS. Blood-free, homogenized, liver cell CL tripled in old age.

These findings suggest that LPS was a pro-inflammatory stimulant, particularly in the middle-aged and old rats. Interestingly, this effect of LPS appears to eliminate many age and gender effects noted in the untreated animals. The observation that a single I.P. injection of LPS has no effect on WBC CL during the first three hours and increases CL during hours 3-48 in all but the old female group, and that the old female rats' WBC CL was depressed during the first 12 hours and enhanced at hour 48, may explain some of the conflicting observations made by others using shorter protocols.

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CHAPTER 1

INTRODUCTION

Gerontology - the study of normal aging.

Approximately thirty million people in the United States of America were over the age of 65 in 1990 (about 12% of the population), (Merck et al., 1990), about two-thirds of whom were female (Kane et al., 1989). This 12% of the population utilized more than 30% of all the health care resources (Merck et al., 1990). It is projected that by the year 2030 20% of the population will be aged and their health care usage will increase to over 50% of the total (Kane et al., 1989; Merck et al., 1990). When 20% of the population utilizes 50% of the resources, and the access is largely supported by public money (Medicare, Medicaid and social security), aging becomes a social as well as a public health problem.

Studies in gerontology and geriatrics, Public Health and medicine must be intensified. By the time a person reaches the eighth or ninth decade, the individual has undergone a number of progressive aging changes that, if not recognized as normal, can raise some troublesome diagnostic problems and result in unnecessary medical treatment. For example the criteria for physiologic and pathologic autoimmunity are now being re-defined in light of new findings. What were

once considered to be pathologic lymphocytes have been found to be present in many normal people (Rose and Bona, 1993). This is not to say that normal senescent changes are independent of time-related pathological events, but the pathology may or may not be inevitable and universal and therefore, these two aspects should be evaluated separately.

The changes associated with aging begin in young adulthood (about age 30) and are the result of the progressive loss of approximately 1% per year in organ and system function (Kane et al., 1989). These losses do not become clinically significant until multiple organs and systems are involved (Kane et al., 1989).

One of the problems the clinician and researcher face is the lack of knowledge regarding what constitutes normal aging. Biomedical animal research has primarily been done on young male animals (Danh et al., 1983; Whitin and Cohen, 1988; Chang et al., 1988; Moldauer et al., 1989; Ulich et al., 1989; Uysal et al., 1989; van der Poll et al., 1990). Little research has been done on young (Sasada, 1983) or old male and female animals (Lavie and Gershon, 1988; Knook and Brouwer, 1989) and no one to date has looked at the long term effects of *E. coli* LPS. Ideally studies should be designed in such a way as to compare different age groups of males and females. Many older studies compare young and old cohorts which are comprised of both sexes. The latter method would mask male-female differences which may be very

important when attempting to identify the inter-gender variability that allows more female individuals to survive into old age while males succumb. In this research the genders were studied independently to isolate any male and female differences.

Rudolph Virchow (1821-1902) asserted that the changes in bodily function that we interpret as disease are the result of damage to cellular structure and function (Ryan and Majno, 1977). The free radical theory of aging hypothesizes a progressive, gradual decline in organ and system function due to cellular dysfunction resulting from the accumulated effects of free radical alteration of cellular components (Southorn and Powis, 1988). The primary result of free radical production is damage to subcellular components of the cell which then lead to alterations in cell function (Rikans and Moore, 1988; Southern and Powis, 1988). The phagocytes, macrophages and polymorphonuclear leukocytes (PMNs) produce free radicals, complement and cytokines as an anti-microbial, anti-bacterial (gram-negative and gram-positive) immune response in defense of the host (Allen, et al., 1972; Babior, 1978; Samuni et al., 1988; Southorn and Powis, 1988). Free radicals damage both the cells that produce them and those of tissues in close proximity to the release (Le and Vilcek, 1987).

Free radicals can damage tissues by a number of molecular actions (Cadenas, 1989). Alteration of protein

structure, due to the oxidation of sulfhydryl bonds, leads to structural abnormalities as well as decreases in both enzymatic activity and release (Le and Vilcek, 1987; Cadenas, 1989). Polysaccharide depolymerization could alter the structure of cellular receptors used for cell-cell recognition (a possible mechanism for autoimmunity) (Kitohora et al., 1988; Rose and Bona, 1993). Lipid peroxidation can alter the structural integrity of sub-cellular organelles, e.g., lysosomes, vesicles and the plasma membrane (Southorn and Powis, 1988). Alteration of nucleic acids result in base hydroxylation, nicking, cross linking and scission of DNA strands causing mutation, and inhibition of protein, nucleotide and fatty acid synthesis (Southorn and Powis, 1988). Free radicals have also been implicated in the intracellular accumulation of aging pigments such as lipofuscin (Rikans and Moore, 1988). Most of the damage to the cells' cytosol (of both the phagocyte and surrounding tissue) is due to the lipid peroxidation of unsaturated fatty acids during the disposal of phagocytosed antigen (Le and Vilcek, 1987).

Not only can the accumulated effects of free radical production change cellular function throughout life (Lavie and Gershon, 1988; Knook and Brouwer, 1989), they ultimately result in a decrease in the individual's ability to cope with environmental stresses, such as micro-organisms, trauma, etc., and increase the probability of death (Horan

and Brouwer, 1990). During the years 45-75, human males have a higher mortality rate (Siegel, 1976), but females have an increased incidence of autoimmune inflammatory joint disease (Knook and Brouwer, 1989). Thus it appears that the female may be better equipped physiologically to survive the environmental stresses, but these survival mechanisms may inflict a higher incidence of chronic illness.

There are many disease processes which have increased incidence with advancing age, e.g., rheumatoid arthritis (Leff et al., 1983), cerebral vascular accidents, heart disease, and Alzheimer's disease (Kane et al., 1989). Current research evidence indicates that a component of these disease processes is the immune system's disposal of either foreign invaders (such as bacteria, viruses, fungi, etc.) or damaged tissue (the result of infarction, immune complex deposition, chemical injury, etc.) by macrophage and PMN phagocytosis (Ryan and Majno, 1977; Issekutz and Issekutz, 1988).

The aging immune system

The free radical hypothesis is only one of numerous hypotheses of aging. The production of free radicals by the WBC and the free radical's apparent ability to damage cellular components may tie this hypothesis to the cellular aging, error catastrophe, waste product, genome-based, wear-and-tear and autoimmune hypotheses of aging (Southorn and

Powis, 1988). Blood is the only tissue that circulates and, therefore, has access to virtually every part of the body. Even in areas where blood is physically excluded (e.g., by the blood-brain barrier) the macrophage (microglial cells) and their cytokines, still have access (du Molin, 1985).

The monocyte/macrophage are the primary white blood cells responsible for antigen presentation (Akiyama et al., 1988) to the immune system via a process named *phagocytosis* (cellular eating) by the Russian biologist Elie Metchnikoff in 1882 (Ryan and Majno, 1977). Metchnikoff also pointed out that the purpose of the inflammatory response was to attract the phagocytes to the infected or injured area (Ryan and Majno, 1977). The primary antigenic and anti-microbial immune response is the production of free radicals by the monocyte/macrophage [primarily superoxide (O_2^-), from oxidized dioxygen] (Michelson et al., 1977; Allen, et al., 1980; Johnston, 1983) and the PMN [hypochlorite ($Cl_2O_2^-$) utilizing the enzyme myeloperoxidase and oxidizing the halogen chloride] (Sagone et al., 1976; McCord, 1983). This response is known as the respiratory burst because it requires a large increase in cellular oxygen uptake. During the free radical response, not only are the bacteria, their lipopolysaccharides, and damaged tissue eliminated, but often healthy surrounding tissue may be damaged by the defense process (McCord, 1983; Weiss, 1989; Goris, 1992).

Although the innate and acquired immune responses have been systematically studied for many years, no definitive age differences have been documented. In fact, there are conflicting reports regarding the free radical response to common antigens such as *E. coli* LPS (McCord, 1983; Lavie and Gershon, 1988; Uysal et al., 1989). For example, depending on the antigenic challenge under consideration, free radical production has been shown to increase (Lavie and Gershon, 1988), decrease (Davila et al., 1990), and remain unchanged (Lavie and Gershon, 1988) with advancing age. It was in the result of the study by Davila et al., that it became apparent that when rats are raised in a pathogen free environment that the free radical production in response to opsonized zymosan or *Salmonella typhimurium* or Phorbol 12-myristic 13-acetate (PMA) decreased with age. When rats are raised in an open environment and exposed to bacteria and bacterial LPS the response to PMA stimulation is different. For example, peritoneal macrophages (harvested from senescent female C57BL/6J mice five days post intraperitoneal injection of 2.5 ml sterile Brewer's thioglycollate) were reported to increase free-radical production 2-fold when stimulated by zymosan and latex beads, or following PMA stimulation (Babior, 1988). Kupffer cells from senescent rats also show an increase in free radical production in response to PMA (Wake et al., 1989).

Exposure to *E. coli* in the air, water and food accounts for the presence of this bacteria in the mammalian gut flora. The release of *E. coli* LPS from the gut has been implicated in clinical conditions as diverse as acute burns (Deitch, 1990), shock (Knook and Brouwer, 1989; Goris, 1990), peritonitis (Skau and Tenger, 1986; Olofsson et al., 1986), aseptic systemic inflammatory responses (Deitch, 1990; Goris, 1990) and rheumatoid arthritis (Cromartie et al., 1977; Goldenberg et al., 1983; Leff et al., 1983; Goldenberg and Reed, 1985; Skau and Tenger, 1986; McCarthy et al., 1992; Peichl et al., 1992). One proposed route for LPS access to the systemic circulation is transmural migration through the intestinal wall into the peritoneum (Skau and Tenger, 1986). Minor leaks of *E. coli* LPS into the peritoneal cavity are handled by the resident peritoneal macrophages (Skau et al., 1986) and therefore would not elicit overt symptoms, since the antigen would not reach the liver or systemic circulation. More substantial leaks, which overwhelm the local defenses would be seen systemically and would trigger a systemic immune response. Olofsson, Nylander and Olsson (1983) showed that *E. coli* LPS clearance from the rat peritoneal cavity is primarily due to the first pass through the liver and secondarily via the lymphatic system which provides *E. coli* LPS access to the general circulation via the thoracic duct. Once in the systemic circulation, the potential exists for the *E. coli*

LPS to stimulate circulating monocytes (Sagone et al., 1976; McCord, 1983) and secondarily recruit and stimulate peripheral blood PMNs (Klebanoff, 1970; Klebanoff and Pincus, 1971; Sagone et al., 1976; Klebanoff, 1982; McCord, 1983) to mount an acute immune response, which should be over in one to two days. However, Freudenberg, Freudenberg and Galanos (1982) found that the liver releases small amounts of the cleared LPS over a three week period, so there is a distinct possibility that this re-released LPS (Freudenberg et al., 1984) may lead, subsequently, to chronic inflammation.

Hypotheses

HYPOTHESIS 1: Davila et al. (1990) studied young and old rats raised in a pathogen-free environment and found that the old rat macrophage free radical production decreased 75% with age. However, rats raised in an open environment would be exposed to *E. coli* LPS. If there are small transmural leaks of LPS into the peritoneal cavity, and the LPS is cleared through the lymph and liver, then both the liver cells and circulating WBCs of the middle-age and old rats should be primed and have an increased response to Phorbol 12-myristic 13-acetate stimulation and show other age dependent changes in the immune system cells.

HYPOTHESIS 2: Even among rats, females live longer than males. Because the female rats live longer than the males they may age more slowly; if so, old female rats should show less of a difference from young females than old male rats do from young male rats.

Methods

Research plan

The primary aim of this research is to use the whole animal model (young, middle-age and old male and female Wistar rats) to determine whether there are progressive gender or age related changes in the acute and long-term physiologic response to an immunomodulating, intraperitoneal (I.P.), dose of *E. coli* LPS compared to an equal volume of sterile 0.9% sterile saline (vehicle), in particular, the long-term systemic cachectic and inflammatory responses that may contribute to the increased morbidity and/or mortality seen in the elderly population. In order to study the WBC free radical responses in vitro, blood was obtained by lancing the tail vein.

Animals

A total of seventy-nine rats were studied. The study defined three age groups: 2-5 months as the young (male n=15, female n=13), 9-15 months as the middle-aged (male n=14, female n=18), and 24+ months as the old (male n=3, female n=12) group (Cartee, 1994). Seven middle-aged rats (male n=1, female n=6) were used in the control studies (APPENDIX D). The rats were bred and raised in the University of Hawai'i Laboratory Animals Services facility in the Bio-medical Sciences building at the University of

Hawai'i at Manoa. The rats were fed the standard laboratory chow which was supplemented by plain, air-popped popcorn (APPENDIX A). The rats were also allowed to exercise daily (Cartee, 1994), ad lib, for at least one-half hour at 5:00 A.M. (APPENDIX A). The exercise time corresponded to the time the protocol would start, so that the rats would become accustomed to being active at a time of day when they would normally be less active or asleep. The rats were randomly selected from this colony and assigned to the test (LPS in saline) and control (saline) injection protocol groups.

Blood was obtained by lancing the rats' tail vein. The tail lance procedure appeared not to be painful. General anesthesia was not administered, because it would have been impractical for a long term study (El-Maahem and Fletcher, 1981) as anesthesia is itself stressful and alters the blood oxygen concentration. A decrease in the circulating oxygen concentration may alter the respiratory burst response of the white blood cells (Smith and Mohideen, 1991). Because acute and/or chronic stress results in the suppression of the immune response (probably via cortisol release from the adrenal gland) the rats were awake and completely unrestrained sitting in the experimenter's lap (Farris and Griffith, 1949; Goodwin et al., 1981), during the sampling portion of the experimental procedure. Simply exposing the rats to a novel environment (an open field) can produce an increase in core body temperature that is very similar to

LPS induced fever (Morrow et al., 1993). Therefore, this work depends on having the animals highly conditioned to their surroundings and to accept human handling (APPENDIX A). Once the animals were accustomed to the experimental surroundings and personnel, the experimental protocol began.

Prior to the injection, a baseline measurement of each variable was made on all, as yet, untreated rats. This measurement was used for the determination of the effect of age and gender (Chapter 1) and is also included in the Treatment chapter (Chapter 2) as the "time 0" data point.

Both the LPS and saline treated rats were fasted during the initial 24 hours of the protocol. The restriction of food intake by the saline treated rats served as a control for the effect of the cachectic response seen post LPS, i.e., a decrease in food intake of more than 90% during the first day (Goodrick et al., 1983; Moldauer et al., 1989).

In this study, the effects of an immunomodulating I.P. dose (0.5 mg/kg) of *E. coli* LPS (O55:B5 TCA extraction, Sigma Chemical Co.) are compared to the effects from an equivalent volume (1 cc/kg) of the LPS vehicle 0.9% sterile saline (control).

Body temperature [measured to the nearest 0.1 °C with a YSI Tele-thermometer and a 402 series small animal rectal probe, (YSI, Yellow Springs Instrument Co., Inc.)] and body weight [measured to the nearest 0.1 gm with a 700 series Triple Beam Balance (OHAUS, Florham Park, NJ)] data were

measured daily starting two days prior to the initiation of the treatment. On the day of injection (day zero), body temperature and weight were measured prior to the injection (time zero) of LPS or vehicle; post-injection measurements were done at half-hour intervals for 3 hours, hourly through 6 hours, every three hours through hour 12, and daily thereafter through day 24. Blood was sampled beginning at the pre-injection (time 0) and post-injection at hours 1.5, 3.0 and 12, and on days 1, 2, 6, 13, 20 and 24.

To collect peripheral blood the tip of the pre-washed and sterilized tail (using Betadine solution) was cut using a sterilized (alcohol swab) sharp instrument, without crushing or cutting the tail ligament, pre-injection (time 0) and post-injection on days 1, 2, 6, 13, 20 and 24. Tail cuts were kept clean, no antibiotic or dressings were used and no infection occurred in any animal (APPENDIX A). Day zero blood was repeatedly sampled, without re-cutting the tail by removing the blood clot with a sharp instrument. No attempt was made to re-establish blood flow by squeezing or warming the tail using a wet towel, as this distressed the rats. Subsequent days' samples required re-cutting the tail and, when done carefully, the rats did not appear to be stressed (Appendix A).

In order to monitor the functional changes occurring in the white blood cell (WBC), peripheral blood was analyzed for: 1) WBC mobility (mm/min.) in a heparinized glass

capillary tube placed horizontally for five minutes following centrifugation for 5 minutes in a Adams Autocrit Centrifuge (Clay-Adams Inc., New York), 2) WBC counts determined using a hemocytometer following red blood cell (RBC) lysis, using an Becton Dickinson Unopette Test 5856, and 3) in vitro whole blood free radical CL before and following stimulation. CL results are reported as counts per minute per WBC (methods are discussed in the next section).

Whole blood chemiluminescence

All chemicals were obtained from Sigma Chemical Company (St. Louis, Mo.) and equipment from Fisher Scientific Products, unless otherwise stated.

A liquid scintillation analyzer (Tri-Carb 1600CA, Hewlett Packard) set in the out-of-coincidence mode [only one photomultiplier tube operative (Johansen et al., 1983)], was used to measure the chemiluminescence produced by the WBCs in the peripheral blood (Cheson et al., 1976). Because the light emission by phagocytes under native conditions is weak, Luminol was added to all solutions used for the suspension of the blood cells, to a final concentration of 0.142 $\mu\text{g/ml}$ phosphate buffered saline (PBS) to increase the efficiency with which the light is detected when fewer than 10^7 cells are present (Briheim et al., 1984). Phorbol 12-myristic 13-acetate (PMA) is a highly lipophilic compound

from the croton plant that freely passes through the plasma membrane to maximally stimulate protein kinase C (Cheson et al., 1976). In this study, PMA is used as a probe to stimulate maximal free radical production by the WBCs of untreated rats (Chapter 1) and during the time-course study following saline or LPS injection (Chapter 2). All blood samples were handled identically, according to the following protocol.

Blood collection was performed in a room at 21-23 °C with the overhead fluorescent lights turned on. Blood was collected from the tail in a heparinized glass capillary tube pre-marked at 25 mm (μ l). The 25 μ l of blood was then transferred into 475 μ l of 4°C PBS/luminol mixture ([pH = 7.4], hereafter referred to as the peripheral blood mixture) in a plastic, 1.5 ml Snap Seal microtube (West Coast Scientific, Inc., Hayward, CA) prepared the night before and stored at 4°C; the capillary tube was repeatedly flushed with the peripheral blood mixture until no blood residue was visible on the walls of the tube. The microtube of peripheral blood mixture was immediately placed in a covered ice bucket in a windowless, darkened room until analyzed. All further handling of the blood was done in a darkened room at 21-25°C (any data collected at higher room temperatures were excluded). Blood was not tested at physiologic rectal temperature (37.5 °C) because the PMA

effects are rapidly neutralized at temperatures near 37°C (Johansen et al., 1983).

Each test microtube was held in the hand for one minute to re-warm the peripheral blood mixture following its removal from the ice. The peripheral blood mixture was gently re-suspended and warmed further by drawing-up and slowly dispensing 200 μ l samples (repeated three times) using a large orifice pipette tip (Cell Saver tip for fragile cell dispensing, USA/Scientific Plastics, Ocala, FL) on an 200 μ l Gilson Pipetman (France). 200 μ l of the re-suspended mixture was dispensed into a dark-adapted 5 ml polypropylene all purpose, capped, scintillation vial (USA/Scientific Plastics, Ocala, FL) and placed in the varisette (small sample holder) of the scintillation counter. The varisette was then advanced and the vial was lowered into the counting chamber.

The background light emission from the dark-adapted 5 ml plastic, capped, scintillation tube (USA/Scientific Plastics, Ocala, FL) containing a 200 μ l aliquot of peripheral blood mixture was recorded at 30 second intervals for 5 minutes and the mean of the last four recordings (minutes four and five) was subtracted as background from the stimulated cell peak CL (method follows). The scintillation tube was removed from the counter and 1.0 ml of the PMA mixture [1.0 μ g PMA/ml buffer mixture (6.6 ml

RMPI-1640 buffer + 3.4 ml luminol (170 $\mu\text{g}/\text{ml}$ in PBS) was pipetted (1000 μl Gilson Pipetman) slowly down the wall of the scintillation tube into the blood mixture. The tube was returned to the varisette in less than ten seconds, and the varisette was advanced and the tube lowered into the counting chamber and counting was resumed. The total elapsed time was no more than 20 seconds from the time the tube was ejected from the counting chamber until counting was resumed. The counts were again recorded at 30 second intervals until the peak of the respiratory burst was reached and had begun to decay.

WBC chemiluminescence control studies

To determine the effect of changes in blood cell counts on CL (Smith and Mohideen, 1991) during the leukopenia and leukocytosis, two control studies were performed. The first involved: a) decreasing the cell counts by serial dilution of the blood samples, and b) increasing the cell concentration by increasing the blood volume from 25 μl to 50 μl and 100 μl , respectively, placed in the 475 μl PBS/luminol. The second study involved the variation in the concentration of PMA in the RMPI/luminol/PBS mixture from .1 μg to .2 μg and .3 $\mu\text{g}/\text{ml}$ to determine the effect an increase in PMA and luminol concentration per cell would have on the peak CL (for results, see Appendix D).

Other blood measurements

The red blood cell sedimentation rate was monitored as an indicator of the inflammatory effects on RBC-plasma interactions (Reinhart, 1988). Because multiple repeat measurements were made on the same rat, it was not feasible to use the common methods of determining erythrocyte sedimentation rate (ESR). These methods require at least 100 μ l (Wintrobe Tube or Seditainer) to 200 μ l (Westergren Tube) of blood per tube (Patton et al., 1989). Therefore, to minimize blood loss, ESR was determined by collecting 40-50 μ l of whole blood in a heparinized capillary tube. One end of the capillary tube was sealed and placed in Critoseal in a vertical position. Measurements of the ESR were made at the end of 60 minutes (mm/hr). This method has been standardized by comparison to the Wintrobe tube method at various packed cell volumes (PCV) (APPENDIX C) by the Laboratory Animal Services of the University of Hawai'i at Manoa. Since the size of the blood samples varied slightly, the ESR is reported as mm/hr/mm³ blood.

Packed cell volume (hematocrit) was determined by collecting 40-50 μ l of peripheral (tail) whole blood in two heparinized capillary tubes, sealing one end with Critoseal and centrifuging the tubes for five minutes in an Adams Autocrit Centrifuge (Clay-Adams, Inc., New York). The RBC

plus WBC percentage of the total volume was determined for both tubes and an average reported.

The protein concentration of the plasma was determined using a plasma refractometer. By scoring the glass and snapping the tube above the cell layer, the plasma layer from one of the centrifuged capillary tubes could be extruded using a 14 gauge needle (Becton Dickinson and Company, Rutherford, NJ). The syringe had a section of polyethylene tubing attached so that a seal could be made with the capillary tube. Plasma was placed on a hand held plasma refractometer (Atago Co., Ltd., Japan). This refractometer has a precision of ± 0.2 g/dL and a range of 0.0 to 12.0 g/dL.

Post-mortem data collection

On day 24 the animal was anesthetized by injecting, I.P., 30-60 mg/kg Na+ pentobarbital (APPENDIX A). When the rat no longer had a deep tendon reflex, as tested by pinching the foot pad, the abdominal cavity was opened and the intestines were displaced to expose the abdominal aorta. The membrana abdominis (parietal peritoneum) was removed from the aorta using a Kim-Wipe. Exanguination was performed by inserting a 22 gauge needle (Becton Dickinson and Company, Rutherford, NJ) into the abdominal aorta and drawing up the blood into a 10 cc plastic syringe (Becton Dickinson and Company, Rutherford, NJ). Once the heart and

lungs ceased to function, the thoracic cavity was opened and the ribs were retracted to expose the heart and lungs. The parietal pleura and the pericardial sac were cut to expose the organs. The right superior and middle lobes of the lung and the entire heart were removed. The right superior lung lobe and the right ventricle and atria were stored in Bouin's solution (APPENDIX B) for future histological studies. The right middle lung lobe and left ventricle (trimmed of all right ventricle and atrial tissue) were used for their respective wet:dry weight ratios. Tissue weights were measured using a Mettler Type H6 balance (Mettler Inst. Corporation, Hightstown, NJ; precision is to the closest 0.1 mg) as an indication of cellular infiltration and fluid exudation due to a possible systemic inflammatory response. Tissue dry weights were obtained after drying to a constant weight at 110°C for two days (Ryan and Majno, 1977).

The whole liver was removed and weighed. The right lobe was sectioned for 1) determination of the wet:dry weight, and 2) preservation in Bouin's solution. The quadrate lobe was excised and the sinusoids were perfused with 4°C phosphate buffered saline (PBS) via the medial segmental hepatic artery, until no color was visible in the solution exiting via the medial segmental hepatic vein and the liver had changed from a cherry-red to a pale milky brown color. The perfused quadrate lobe was sectioned for 1) wet:dry weight, 2) preservation in Bouin's solution, and

homogenization in PBS for liver CL determination. The sections for wet:dry weight and homogenization were weighed (as above for heart and lung) to the nearest 0.1 mg. The section for wet:dry ratio was placed in an incubator set at 110 °C to dehydrate for 48 hours (Ryan and Majno, 1977), and then re-weighed using the same Mettler balance.

The liver contains more phagocytes (macrophages and Kupffer cells) than any other organ in the body and is the major phagocytic barrier of the body (Wake et al., 1989). A section of the perfused quadrate lobe, to be used for liver cell CL, was weighed and homogenized in a plastic vial in PBS. The volume of PBS was determined by the equation: PBS volume (cc)=15 x liver wet weight (gm). The liver section was homogenized for one minute using the Tissue Tearor (Biospec Products, Bartlesville, Oklahoma) set at 4000 rpm. One milliliter (ml) of the homogenate was placed in a 1.5 ml plastic, capped, centrifuge tube (USA/Scientific Plastics, Ocala, FL) and spun for five minutes in a table top centrifuge. The pellet was visually examined for RBC and graded as follows: 0 (no visible RBC), +1 (scattered RBC, but no aggregation), +2 (< 1 mm aggregation), +3 (> 1 mm aggregation RBC). Any pellet with a score of \geq +2 was deleted from the study due to residual blood cell contamination. Neither age, gender nor prior treatment had any consistent effect on residual blood cell contamination. 200 μ l of the homogenate was used to determine liver CL

using the same procedure as that for whole blood CL (see above). 200 μ l of the homogenate was used to determine the liver dry weight (mg). The liver chemiluminescence was expressed as counts per minute per milligram dry weight.

Statistical analysis

Inter-age and gender effects were analyzed using a one-way analysis of variance (ANOVA). The interaction between age and gender, age and treatment, and gender and treatment was analyzed using a two-way ANOVA . Treatment effects were analyzed using a one-way ANOVA and the Student's t-test. The interaction between age, gender and treatment was analyzed using a three-way ANOVA. The statistical program used was SYSTAT for Windows, version 5.03.

AGE AND GENDER RESULTS

An analysis of variance by age on untreated rats shows that there was a statistically significant increase in WBC chemiluminescence (CL) with age in male rats (Fig. 1.1.1) (ANOVA, $p < .001$) with a 2-fold increase taking place between young and old (t-test, $p < .001$), and a 1-fold increase between middle and old age (t-test, $p < .010$). Female rats show a similar response (Fig. 1.1.2) (ANOVA, $p < .001$) with a 1-fold increase between middle and old age (t-test, $p < .001$) and a 3-fold increase between young and old age (t-test, $p < .001$). Males were not statistically different from the females at any age (t-test, $p < .093$).

Male rats show a decrease in time-to-peak (TTP) CL (Fig. 1.2.1) during middle-age (ANOVA, $p < .001$; t-test, $p < .007$), but TTP increased between middle and old age (t-test $p < .005$). However, there was no difference in TTP between young and old age in male rats (t-test, $p < .245$). The female rat's (Fig. 1.2.2) WBC TTP does not show a difference between young and middle-age (t-test, $p < .096$) or middle and old age (t-test, $p < .085$), rather TTP steadily increases with advancing age achieving statistical significance between young and old rats (t-test, $p < .018$). Young male and female rats' WBC TTP is statistically different (t-test, $p < .015$) while there are no gender differences in middle (t-test, $p < .117$) and old age (t-test, $p < .232$).

Age is associated with a steady decrease in the circulating WBC counts (ANOVA, $p < .043$) in male rats (Fig. 1.3.1), with about 19% of the decrease occurring by middle-age (t-test, $p < .017$) and total decrease of 35% by old age (not significant, t-test, $p < .119$). The female rats' WBC counts (Fig. 1.3.2) also decrease (ANOVA, $p < .043$), but a 27% decrease has occurred by middle-age (t-test, $p < .002$) and a total decrease of 35% has occurred by old age (t-test, $p < .009$). There are gender differences in the young (t-test, $p < .005$) and middle-age (t-test, $p < .001$), but the difference disappears in old rats (t-test, $p < .365$).

Age is associated with a linear decrease in WBC mobility (ANOVA, $p < .009$) in the male rat (Fig. 1.4.1). Although the decrease is not statistically significant, the WBC is about 50% less mobile in middle-age (t-test, $p < .138$) and has zero mobility in old male rats (t-test cannot be done when the mean is zero). The female rats' WBC mobility (Fig. 1.4.2) also decreases (ANOVA, $p < .009$), but the 67% decrease in middle-age is not statistically significant (t-test, $p < .051$); there is a further decrease in old age, but the females' WBCs remain mobile in old age (t-test, $p < .039$). Statistically, males and females were not different at young (t-test, $p < .121$) and middle-age (t-test, $p < .475$). The fact that the mean WBC mobility in the old male rat ($n = 3$) was zero seems biologically significant, although a t-test

cannot be done since the mean was zero and therefore had no standard deviation.

Age is associated with a 13% increase in the circulating plasma protein (PP) concentration (ANOVA, $p < .001$; t-test, $p < .001$) in the middle-aged male rat (Fig. 1.5.1), and a non-significant 6% increase (for a total of 19%) in the old male rat (t-test, $p < .052$). Female rats show an 8.5% increase in circulating PP (Fig. 1.5.2) in middle age (ANOVA, $p < .001$; t-test, $p < .001$) and a total increase of 13% in old age (t-test, $p < .003$). There are gender differences in middle-age (t-test, $p < .021$), but not in the young (t-test, $p < .724$) or old (t-test, $p < .556$) age groups.

Age is associated with and non-significant increase in PCV in middle-aged males and a decrease PCV (ANOVA, $p < .045$) (Fig. 1.6.1) of about 13% between middle-age and old male rats, but this is not statistically significant by t-test ($p < .825$). The female rats (Fig. 1.6.2) show a 13% increase in PCV (ANOVA, $p < .045$) in middle-age (t-test, $p < .172$), but a 3% decrease, in reference to the young, in old age (t-test, $p < .763$). There is a gender difference between the young and middle-aged (t-test, $p < .010$), but not between the young (t-test, $p < .173$) and old (t-test, $p < .053$) age groups.

Age is associated with a 50% increase in body weight (ANOVA, $p < .001$) in middle-age male rats (Fig. 1.7.1) (t-test, $p < .001$) and remains unchanged in old age (t-test, $p < .309$). Body weight increases 20% in the middle-aged

female (Fig. 1.7.2) (ANOVA, $p < .001$; t-test $p < .001$) and remains unchanged in old female rats (t-test, $p < .001$). There are gender differences in the young (t-test, $p < .001$) and middle-age (t-test, $p < .001$) but there is no statistical difference in old age (t-test, $p < .149$) which is likely due to the small number of old male rats ($n = 3$) and the large standard error of the mean (S.E.M.).

Age is associated with a 90% increase in liver CL in middle-aged male rats (t-test, $p < .004$) and a 3-fold increase between middle and in old age (Fig. 1.8.1) (ANOVA, $p < .012$). Old female rats show a 1-fold increase between young and old age (Fig. 1.8.2) (ANOVA, $p < .002$; t-test, $p < .147$). Male and female rats showed similar liver CL responses when perfused liver cells were stimulated with PMA *in vitro* (Figures 1.8.1 and 1.8.2). The only difference was a further doubling between middle and old-aged male rats, but the small old rat population and the large variation preclude a meaningful analysis (t-test, $p < .390$).

In the rat colony raised for this study, the average gender distribution in the various litters showed that more male rats (mean = 8) were born than females (mean = 6). Although an equal number of 9 young males and female rats were selected from the litters for the old age study, in middle-age an increase in the male rat mortality (6 deaths) resulted in the survival to old age of only three male rats while all 9 female rats survived.

Age and gender had no statistically significant effect on ESR, and rectal temperature. These data are shown in Tables 2.1.1 through 2.6.6.

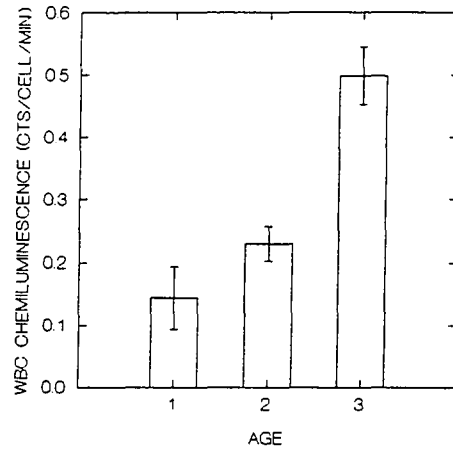


Figure 1.1.1. The effect of age on WBC peak CL in WBCs of untreated male rats following in vitro PMA stimulation (mean \pm S.E.M., $p < .001$). Groups shown are: young (1, $n=15$) and middle-age (2, $n=14$), and old (3, $n=3$).

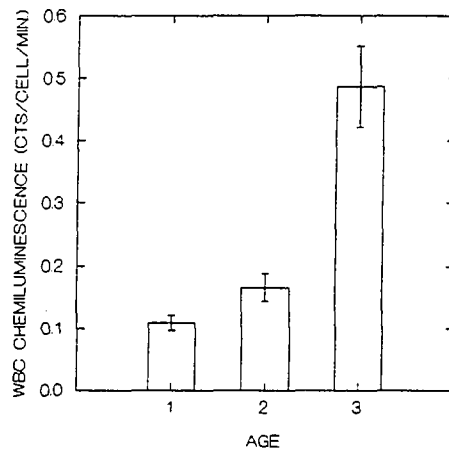


Figure 1.1.2. The effect of age on WBC peak CL in WBCs of untreated female rats following in vitro PMA stimulation (mean \pm S.E.M., $p < .001$). Groups shown are: young (1, $n=13$) and middle-age (2, $n=18$) and old (3, $n=9$). The difference between groups 1 and 2 for both males (above) and females are statistically significant ($p < .011$, by t-test). The independent variables gender and age do interact to affect CL ($p < .001$).

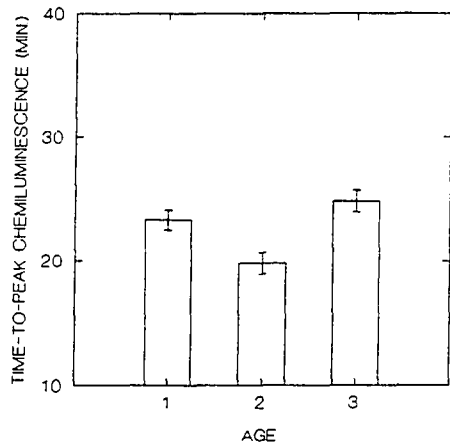


Figure 1.2.1. The effect of age on WBC CL time-to-peak in WBCs of untreated male rats following in vitro PMA stimulation (mean \pm S.E.M., $p < .000$). The groups shown are: young (1, $n = 15$), middle-age (2, $n = 14$), and old (3, $n = 3$).

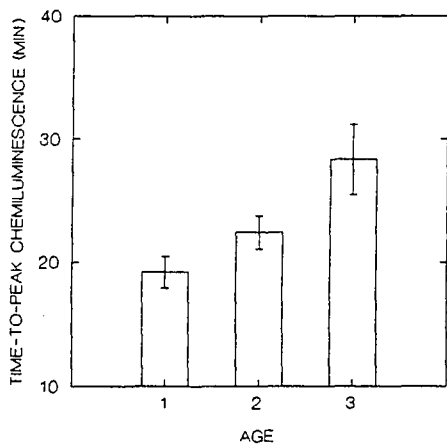


Figure 1.2.2. The effect of age on WBC CL time-to-peak in WBCs of untreated female rats following in vitro PMA stimulation (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n = 13$), middle-age (2, $n = 18$), and old (3, $n = 9$). The independent variables gender and age do interact to affect time-to-peak CL ($p < .001$).

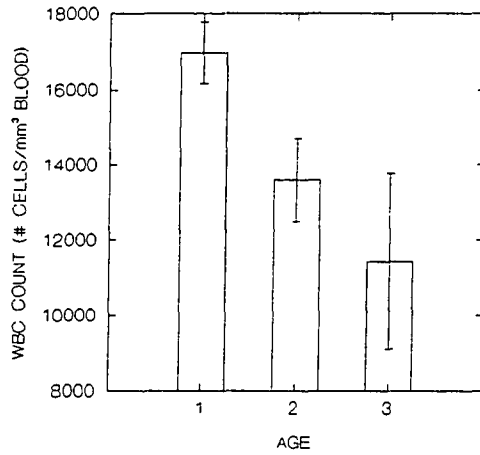


Figure 1.3.1. The effect of age on WBC counts of untreated male rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), old (3, $n=3$). The independent variables gender and age do interact to affect the WBC counts ($p < .043$).

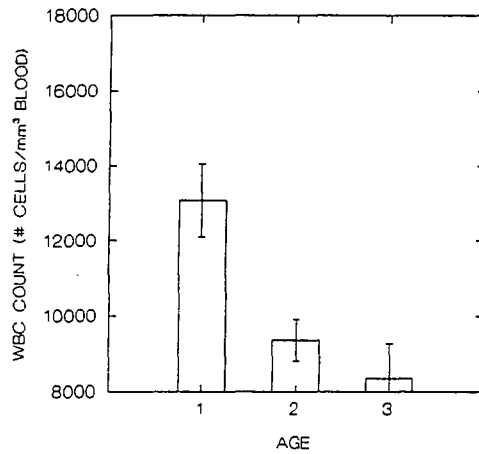


Figure 1.3.2. The effect of age on WBC counts of untreated female rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=13$), middle-age (2, $n=18$), old (3, $n=9$). The independent variables gender and age do interact to affect time-to-peak CL ($p < .001$).

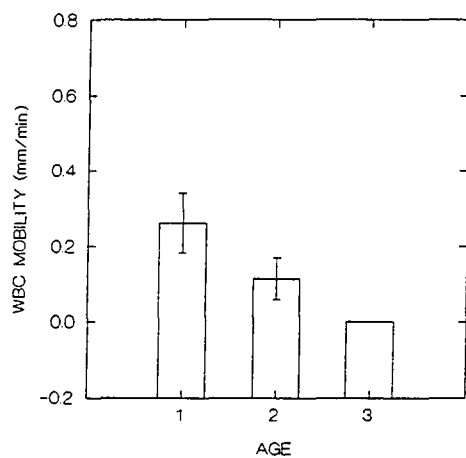


Figure 1.4.1. The effect of age on the WBC mobility of male rats (mean \pm S.E.M., $p < .009$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), old (3, $n=3$).

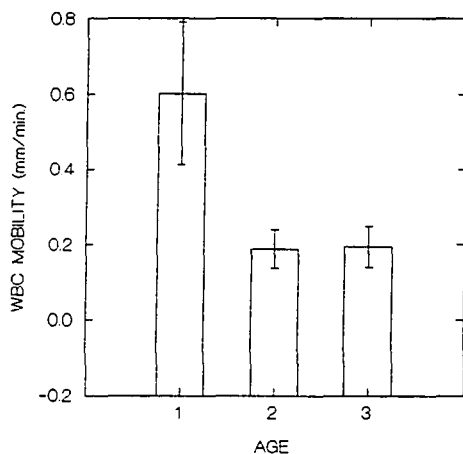


Figure 1.4.2. The effect of age on the WBC mobility of female rats (mean \pm S.E.M., $p < .009$). The groups shown are: young (1, $n=13$), middle-age (2, $n=18$), old (3, $n=9$). The independent variables gender and age do not interact to affect WBC mobility ($p < .296$).

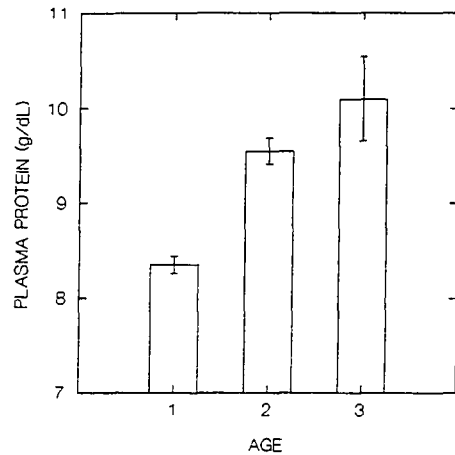


Figure 1.5.1. The effect of age on the plasma protein concentration (g/dL) of untreated male rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$).

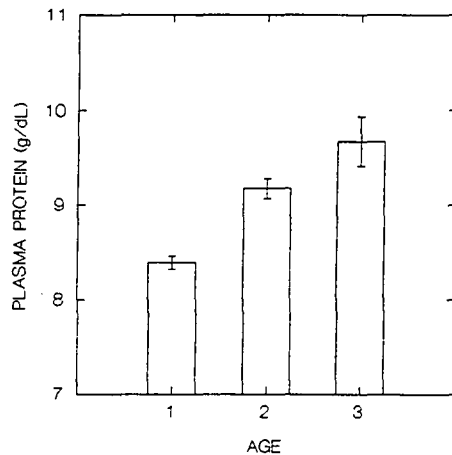


Figure 1.5.2. The effect of age on the plasma protein concentration (g/dL) of untreated female rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=13$), middle-age (2, $n=18$), and old (3, $n=9$). The independent variables gender and age do not interact to affect the plasma protein concentration ($p < .358$).

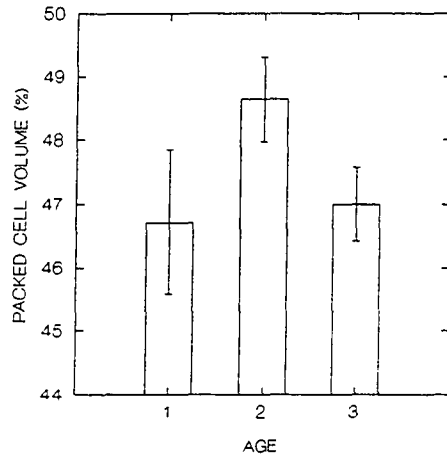


Figure 1.6.1. The effect of age on packed cell volume of untreated male rats (mean \pm S.E.M., $p < .045$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$).

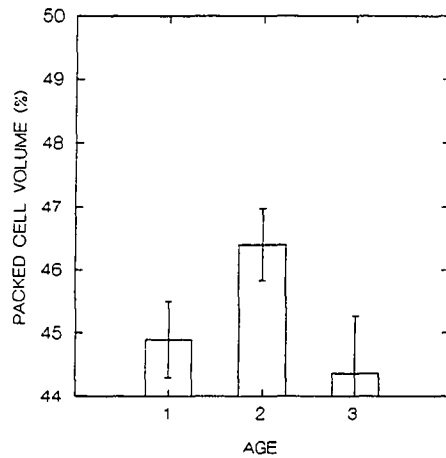


Figure 1.6.2. The effect of age on packed cell volume of untreated female rats (mean \pm S.E.M., $p < .045$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$). The independent variables gender and age do not interact ($p < .099$).

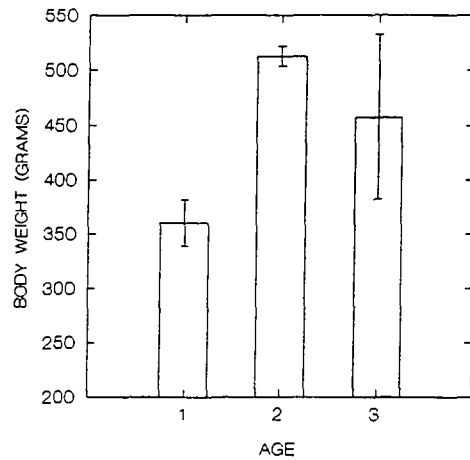


Figure 1.7.1. The effect of age on body weight in male rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$).

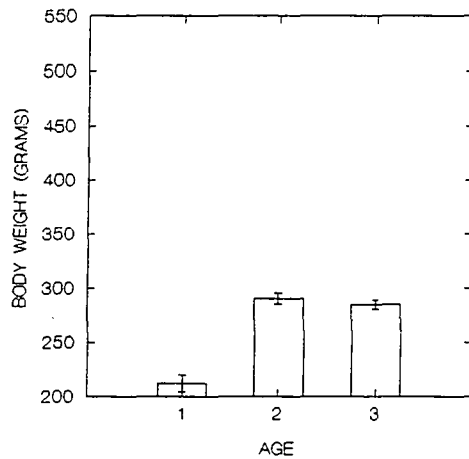


Figure 1.7.2. The effect of age on body weight in female rats (mean \pm S.E.M., $p < .001$). The groups shown are: young (1, $n=13$), middle-age (2, $n=18$), and old (3, $n=9$). The independent variables gender and age do interact ($p < .001$).

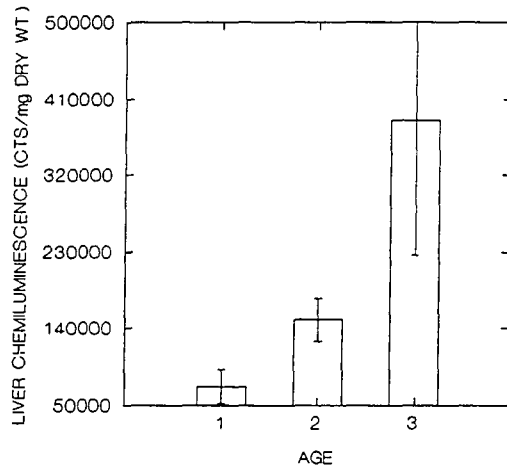


Figure 1.8.1. The effect of age on post-mortem liver CL in male rats, following PMA stimulation (mean \pm S.E.M., $p < .002$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$).

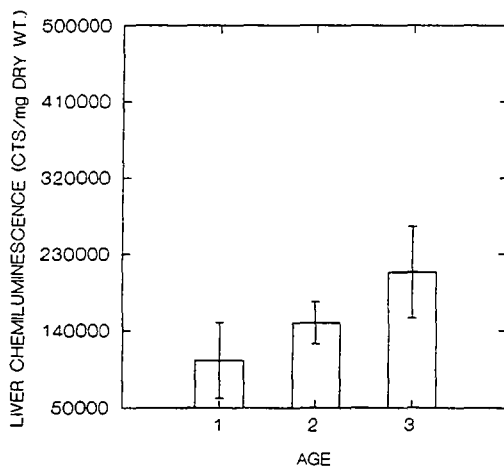


Figure 1.8.2. The effect of age on post-mortem liver CL in female rats, following PMA stimulation (mean \pm S.E.M., $p < .002$). The groups shown are: young (1, $n=15$), middle-age (2, $n=14$), and old (3, $n=3$). The independent variables gender and age do not interact ($p < .099$).

CHAPTER 2

THE EFFECTS OF TREATMENT

Inflammation

Celsus, in the first century AD, put together the fundamental signs of inflammation, i.e., redness (*rubor*), swelling (*tumor*), pain (*dolor*), and warmth (*calor*), known to the Greeks and Egyptians for more than 2000 years BC, to provide modern medicine with a clinical definition of inflammation (Ryan and Majno, 1977). The underlying processes of these inflammatory signs ultimately result in the loss of normal physiological function (*functio laesa*) a fifth sign identified by Rudolph Virchow (1821-1902) (Ryan and Majno, 1977). Julius Cohnheim (1867) provided the explanation for the five cardinal signs of Celsus and Virchow - vasodilatation accounts for the *rubor*, an increase in blood flow causes the *calor*, exudation produces the *tumor*, which in turn irritates the nerve fibers resulting in *dolor* (Ryan and Majno, 1977), while the functional loss can be attributed to changes in water content, soluble mediator release, e.g., cytokine and lymphokines, and their effects on the surrounding tissue (Le and Vilcek, 1987).

The immune system's free-radical response to microbes, bacteria, and LPS is a possible mechanism for the organ and system changes that could be considered normal aging at one level and pathological aging if produced in excess.

Everyone is exposed to gram-negative bacteria from the time they are born. *Escherichia coli* resides in our gut. When this bacterium is viable, its antigenic components, i.e., the lipopolysaccharide (LPS) portion of the cell wall, also known as endotoxin, are sequestered within the outer membrane, hidden from our immune system by the oligosaccharide (O-antigen) portion of the LPS (Morrison and Ryan, 1979; Raetz et al., 1991). When the bacteria die the cell wall disintegrates, releasing LPS and exposing the antigenic determinant Lipid-A to the immune system, and thereby eliciting the inflammatory component of the immune response (Morrison et al., 1985; Raetz et al., 1991).

The vertebrate immune system has two distinct parts - 1) the non-specific (natural or native), and 2) the acquired (specific or reactive) response to foreign substances (antigens). Both mechanisms contribute to producing symptoms such as hypothermia, fever, pilo erection, leukopenia, leukocytosis, etc. (Morrison and Ryan, 1979; Maizel and Lachman, 1984; Le and Vilcek, 1987; Ulich et al., 1989).

Innate immunity is provided by the physiological barriers (skin, mucous membranes), circulating molecules of complement and other specific opsonins (Campbell and Campbell, 1988), natural killer cells, and free radical production by what are called the professional phagocytes, i.e., the polymorphonuclear leukocyte (PMN), and the

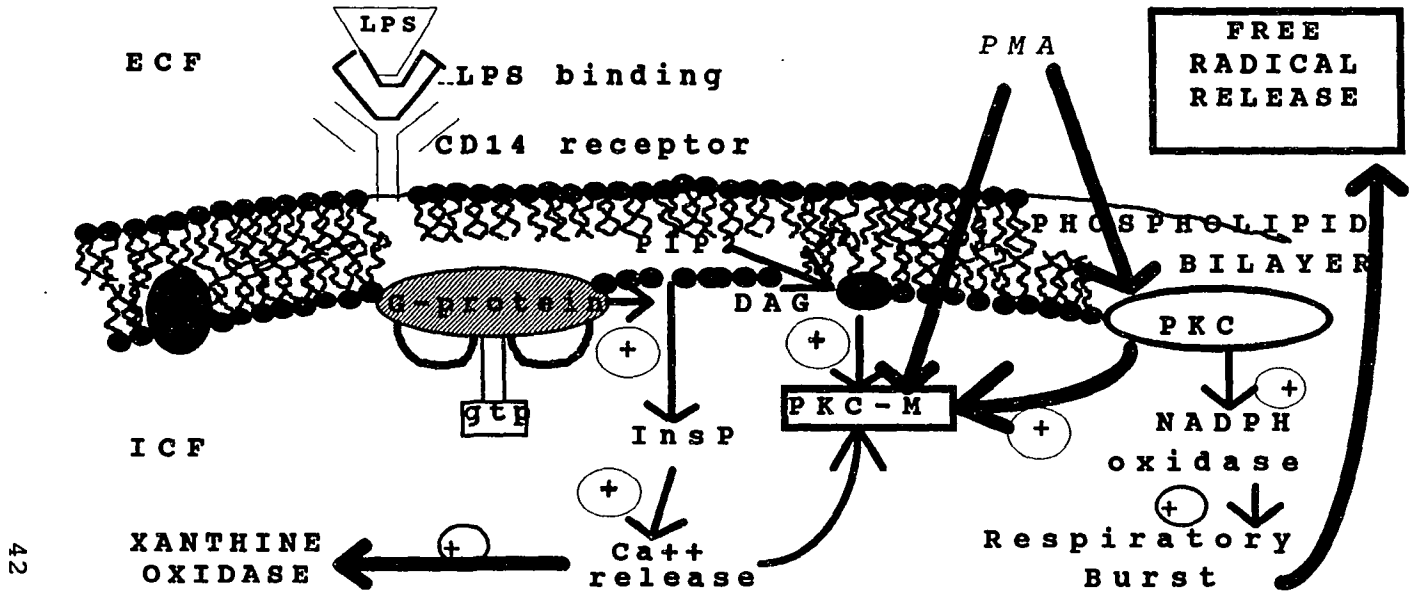
macrophage (Flohe and Gietz, 1987; Akiyama et al., 1988). The macrophage releases soluble mediators that act on other cells, e.g., tumor necrosis factors (TNF- α and - β), and β - and γ -interferon (Dinarello and Mier, 1987; Debets et al., 1988; Ziegler, 1988). The innate system is supported by the acquired response - the lymphocytes and their antibodies and cytokines (Dinarello and Mier, 1987; Akira et al., 1990).

There are two mechanisms for the production of free radicals in the body. First, the mitochondria generate free radicals during the production of adenosine triphosphate (ATP) (Southorn and Powis, 1988). Second, an increase in the oxidative metabolism of phagocytic cells' during phagocytosis (even of inert particles) produces free radicals via a nicotinic adenine dinucleotide phosphate (NADP) dependent chain reaction (Salin and McCord, 1977; Ulich et al., 1987).

At the inflammatory site, the antigen binds to the PMNs' cell surface and triggers their free radical production (primarily hypochlorite ($\text{Cl}_2\text{O}_2^{--}$)) (Paul et al., 1970; McCord et al., 1977; Salin and McCord, 1977; McCord, 1983; Baggiolini and Dewald, 1985; Samuni et al., 1988). To support the increased free radical production, the phagocytes must maintain an increased oxidative metabolism, i.e., increased energy consumption, to maintain their reducing power (Southorn and Powis, 1988). This is accomplished by increased glycolysis and the utilization of

the hexose monophosphate shunt (HMPS) (Paul, 1970), also known as the 6-phosphogluconate dehydrogenase pathway. By converting a hexose to a pentose (a component of ATP, NAD, FAD, CoA, RNA and DNA) the HMPS produces the reducing power (H^+) to regenerate NADPH from NADP thus maintaining the above enzymatic cascade and the phagocyte respiratory burst without involving energy production by the Krebs' (TCA) cycle or the mitochondria (Paul, 1970; McCord, 1983; Sasada, 1983).

The NADPH oxidase is turned on physiologically by the coupling of an antigen and a binding protein to form a complex that then binds to a specific plasma membrane receptor; or, artificially by a molecule such as Phorbol 12-myristic 13-acetate (PMA) (Buys, et al., 1987; Ganong, et al., 1987). PMA is a highly lipophilic molecule that freely penetrates the plasma membrane and is therefore available in large quantities (Moolenaar et al., 1984; Ganong, et al., 1987). Because PMA has a structure similar to diacylglycerol (DAG's) PMA, and is available in high concentrations, it can maximally stimulate protein kinase C (Ganong, et al., 1987; Schultz et al., 1991) and, thus, the NADPH oxidase respiratory burst (Sasada et al., 1984; Lavie and Gershon, 1988), see Figure 2.1 - Phagocyte free radical production (Altman, 1988; Schumann et al., 1990; Raetz et al., 1991).



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Figure 2.1 Phagocyte free radical production (WBC CL).

The activated macrophage recruits T- and B-lymphocytes and thus initiates the humoral or acquired immune response (Le and Vilcek, 1990). In response to the TNF- α and IL-1 secreted from the stimulated macrophage, the T-lymphocyte binds to the MHC-II-antigen complex presented on the surface of the macrophage (Le and Vilcek, 1990). Thus activated the helper T-cell will begin to secrete IL-1, IL-2 and TNF- α . IL-2 is a growth factor for T-cells and stimulates cytotoxic T-cells. INF- γ stimulates further TNF- α release from and class II MHC expression on the macrophage. IL-3 stimulates the hematopoiesis that is responsible for the leukocytosis (Dinarello and Mier, 1987). The cytotoxic T-cell recognition of the antigen-MHC-II complex leaves the macrophage susceptible to lysis, since the cytotoxic T-cell lyses any cell that has both markers on its plasma membrane (Le and Vilcek, 1990).

T-cell adhesiveness is peaking as PMN adhesiveness is waning at 24-72 hours post antigen stimulation (Thornhill et al., 1990). IL-1, TNF and LPS all stimulate leukocyte adhesion to endothelial cells (Thornhill et al., 1990). INF- γ stimulates T-cell, but not PMN adhesion to endothelial cells (Thornhill et al., 1990).

As a part of the non-specific immune response, the activated macrophage releases complement and other opsonins that bind to the circulating antigen. While this detoxifies

the antigen the resulting immune complexes have been shown to become lodged in tissues where the complexes trigger a local inflammatory response (Brandt et al., 1968; Ng et al., 1989).

Immune complexes are also formed by an antibody, such as IgG released from B-lymphocytes, and antigen interaction, and these immune complexes trigger humoral and cellular reactions that involve complement, neutrophils, T- and B-cells (Brandt et al., 1968; Ng et al., 1989).

Delpierre et al. (1980) found a 10-15,000 MW protein(s) in senescent old cow and mice lymphoid cultures, which they call the old cow extract (OCE). In previously sensitized animals, where antigen was injected five days prior to immune challenge with sheep red blood cells, OCE produced an inhibition of the secondary *in vitro* humoral immune response, with an increase in the suppresser cell activity (perhaps non-T-cell) (Delpierre et al., 1980).

The current literature shows evidence that both supports and refutes claims of immunological changes with advancing age. The design of this study in young, middle-aged and old male and female rats is an attempt to simulate the leak of *Escherichia coli* lipopolysaccharide (the antigenic factor of the *E. coli* external cell wall) from the intestine into the peritoneal cavity. This type of leak is suspected in the following clinical conditions: 1) post burn (Deitch, 1990), 2) rheumatoid arthritis (Goldenberg et al., 1983;

Leff et al., 1983; Goldenberg et al., 1985; Skau and Tenger, 1986; McCarthy et al., 1992; Peichl et al., 1992), and 3) inflammatory bowel disease (Kitahora et al. 1988; Mills, 1989) and other disorders of the digestive system (Shamburek and Farrar, 1990). Clearance of *E. coli* LPS from the peritoneal cavity is via two mechanisms, 1) intraperitoneal destruction, which starts immediately (Skau et al., 1986), and 2) absorption and distribution to systemic defense systems via the portal circulation, with uptake by the liver, and via the lymphatic circulation thereby providing *E. coli* access to the systemic circulation (Braude, 1964; Freudenberg, Freudenberg and Galanos, 1982; Olofsson, Nylander and Olsson, 1983; Dunn et al., 1985; Skau et al., 1986). LPS can actually be found within the circulating PMNs (Braude, 1964).

Most of *E. coli* endotoxin that reaches the liver is there for more than 60 days (Braude, 1964). Since aging decreases liver volume, blood flow (Shamburek and Farrar, 1990), *in vitro* Kupffer cell phagocytosis (Knook and Brouwer, 1989) and *in vivo* hepatocyte clearance of LPS (Freudenberg et al., 1982; Freudenberg, et al., 1984), the aged animal may clear less of the LPS during the first pass through the liver (Wake et al., 1989) and thus have a greater percentage of the initial dose of LPS entering the systemic circulation to prime the immune system for a secondary response (accelerated and amplified) to subsequent

exposures to LPS (Knook and Brouwer, 1989). If this is so, the aged and young animals should have a different chronic response to an I.P. injection of LPS. The purpose of this study was to determine: 1) whether the concentration of the *E. coli* antigen cleared via the lymphatic circulation was sufficient to trigger an inflammatory response in the peripheral white blood cell population, 2) what, if any, physiologic changes occur (Smith, 1926) and, 3) if there are long term (greater than 3 days) effects of a single I.P. injection of the *E. coli* lipopolysaccharide (LPS).

Most young animals survive high doses (10-60 mg/kg, I.P.) of LPS , but have chronic sequelae and overt symptoms of arthritis (Cromartie et al., 1976). Other young and the majority of older animals die of multi-organ failure and shock at these dosages (Knook and Brouwer, 1989). It has been my experience that aged (20+ months) rats succumb to I.P. *E. coli* LPS at a dose as low as 1.0 mg/kg. At doses between 0.25 and 1.0 mg/kg LPS (I.P.) all rats survive, and suffer only minor cold/flu-like symptoms (see APPENDIX A). At the 0.5 mg/kg dose, LPS was an effective time-dependent immunomodulator with both pro- and anti-inflammatory effects.

I. LPS treatment: acute phase

A. Body Temperature

In young male rats (Fig. 2.2.1), LPS treatment caused a decrease in rectal temperature (hypothermia) between 0.5 and 3.5 hours with a nadir at 2.5 hours (t-test $p < .001$), followed by fever between hours five and 24, peaking at 6 hours (t-test, $p < .001$). In young female rats (Fig. 2.2.2), LPS increased rectal temperature at the half hour followed by hypothermia between 1 and 3 hours, nadir at 2.0 hours (t-test, $p < .001$) and fever between hours 5 and 24, peak at 6 hours (t-test, $p < .001$).

In middle-aged male rats (Fig. 2.3.1), LPS treatment produced hypothermia between 1 and 4 hours, nadir at 1 hour (t-test, $p < .001$) followed by a fever between hours 6 and 48, peak at 12 hours ($p < .001$). In middle-aged female rats (Fig. 2.3.2), LPS treatment produced hypothermia between 1 and 4 hours, nadir at 2 hours (t-test, $p < .001$), followed by a fever between hours 6 and 24, peak at 6 hours (t-test, $p < .001$).

In old male rats (Fig. 2.4.1), LPS treatment produced hypothermia between 1 and 5 hours, without fever between hours 6 and 48. In old female rats (Fig. 2.4.2), LPS treatment produced hypothermia between 1 and 6 hours, nadir at 3 hours (t-test, $p < .001$) without a fever between hours 6 and 48 ($p < .073$).

Overall, the effects of LPS on rectal temperature were very similar in male and female rats, other than the 30 minute peak in young and middle-age female rats which was absent in the other groups.

B. White Blood Cell Chemiluminescence

In young male rats (Fig. 2.5.1), LPS treatment produced an increase in WBC chemiluminescence (CL) at hour 1.5 with an acute phase peak CL at 12 hrs which was maintained through hour 48 (t-test, $p < .001$). The increase in CL due to LPS was more than 2 times that of the control treatment. In young female rats (Fig. 2.5.2), LPS treatment produced an increase in WBC CL after hour 3 which reached an acute phase peak at 48 hours. The increased CL due to LPS was more than twice that of the control treatment (t-test, $p < .005$) and the male and female responses were similar.

In middle-aged male rats (Fig. 2.6.1), LPS treatment produced an increase in CL at hour 1.5 followed by a slight decrease between 3 and 12 hours then increased again to reach an acute phase peak at 48 hours (t-test, $p < .002$). The increase in CL due to LPS was more than 6 times greater than that of the control treatment at 48 hours. In middle-age female rats (Fig. 2.6.2), LPS treatment produced an increase in WBC CL at hour 3 and reached an acute phase peak CL that was approximately three times greater than middle age control values (t-test, $p < .001$) and twice that of the young female LPS treated rats (t-test, $p < .008$). Middle-aged males

maintain a greater CL response than that of the middle-aged female rats ($p < .001$).

In old male rats (Fig. 2.7.1), LPS produced a phasic response with an initial decrease in CL at 0.5 hours, an increase during hours 1.5 and 3, followed by a decrease at hour 12 and, finally, an acute phase peak CL was reached at 48 hours. In old female rats (Fig 2.7.2), LPS produced a similar phasic response with an initial decrease in CL at 0.5 hours, a return to control values during hours 1.5 and 3, again a decrease at hour 12 with the acute phase peak CL at 48 hours. The peak CL at hour 48 due to LPS, was 4 times greater than old female control values (t-test, $p < .001$). Both male and female old rats were shown to increase CL after LPS more than middle-aged and young rats (t-test, $p < .002$). Generally males and female rat WBC CL responses were similar.

C. Time-to-peak CL

In young male rats (Fig. 2.8.1), LPS treatment produced a decrease in the time-to-peak CL (TTP) between 0.5 and three hours (t-test, $p < .001$) returning to control values at hour 48. In young female rats (Fig. 2.8.2), LPS treatment produced a decrease in the TTP between 0.5 and 12 hours which is not statistically different from control values during the first 48 hours (t-test, $p < .165$).

In middle-aged male rats (Fig. 2.9.1), LPS treatment produced a decrease in the TTP between 0.5 and 12 hours (t-

test, $p < .001$), returning to control values by hour 48. In middle-aged female rats (Fig. 2.9.2), LPS treatment produced a decrease in the TTP between 1.5 and 12 hours (t-test, $p < .001$), returning to control values by hour 24.

In old male rats (Fig. 2.10.1), LPS treatment produced a decrease in the TTP between 1.5 and 3 hours, returning to control values between hours 12 and 24, with a second decrease at 48 hours. In old female rats (Fig. 2.10.2), LPS treatment produced a decrease in the TTP between 0.5 and 3 hours (t-test, $p < .003$), returning to control values by hour 12.

Male and female rats' WBC TTP in response to LPS was very similar at all ages.

D. WBC counts

In young male rats (Fig. 2.11.1), LPS treatment produced a WBC leukopenia between hours 0.5 and three with a nadir at hour 1.5 (t-test, $p < .001$). WBC counts then increased above control values (leukocytosis) at 24 hours (t-test, $p < .061$) and returned to control values by 48 hours. In young female rats (Fig. 2.11.2), LPS produced a leukopenia with a nadir between 1.5 and 3 hours (t-test $p < .002$). WBC counts then returned to, but never exceeded, control values (t-test, $p < .423$).

In middle-aged male rats (Fig. 2.12.1), LPS produced a leukopenia between 0.5 and 3 hours (t-test, $p < .001$) and a leukocytosis over three times greater than control values by

hour 24 (t-test, $p < .001$). The WBC counts began to decrease, but were still above that of the control rats at hour 48 ($p < .009$). In middle-aged female rats (Fig. 2.12.2), LPS produced a leukopenia between 0.5 and 3 hours (t-test, $p < .001$) and a leukocytosis by hour 24 ($p < .001$).

In old male rats (Fig. 2.13.1), LPS produced a leukopenia between 0.5 and 3 hours and a leukocytosis, more than three times greater than control values, by hour 24. In old female rats (Fig. 2.13.2), LPS produced a leukopenia between 0.5 and 3 hours (t-test, $p < .001$) and a leukocytosis, more than two times greater than control values, by hour 24 ($p < .002$).

In general, there were no significant gender differences in the WBC response to LPS, but males did have consistently higher WBC counts (t-test, $p < .001$)

E. Plasma Protein Concentration:

In young male rats (Fig. 2.14.1), LPS treatment produced a decrease in circulating plasma protein concentrations (PP) between 1.5 and 3 hours (t-test, $p < .002$). PP then increased above control values peaking at 24 hours (t-test, $p < .021$). Similarly, in young female rats (Fig. 2.14.2), LPS treatment produced a decrease in circulating plasma protein concentrations (PP) between 1.5 and 3 hours (t-test, $p < .013$). PP then returned to control values during hours 12 and 24 and, unlike the males failed to increase further, but

rather again decreased below control values at 48 hours (t-test, $p < .057$).

In middle-aged male rats (Fig. 2.15.1), LPS treatment produced a decrease in circulating plasma protein concentrations (PP) between 1.5 and 3 hours (t-test, $p < .001$). In middle-aged female rats (Fig. 2.15.2), LPS treatment produced a decrease in circulating plasma protein concentrations (PP) between 0.5 and 3 hours (t-test, $p < .002$), and again at hour 48 ($p < .038$). The latter decrease at 48 hours is similar to young female rats' PP and different from the males' response.

In old male rats (Fig. 2.16.1), LPS treatment produced a decrease in circulating plasma protein concentrations (PP) at hour 1.5 and 3, and an increase at hour 12. In old female rats (Fig. 2.16.2), LPS treatment produced a non-significant decrease in circulating PP between 1.5 and 3 hours (t-test, $p < .329$) and a non-significant increase at hours 12 and 24 (t-test, $p < .212$). This 12-24 hour pattern is different from that of the young rats, but similar to that of the old male rats.

E. Packed Cell Volume

In young male rats (Fig. 2.17.1), LPS treatment produced a decrease in circulating packed cell volume (PCV) between 1.5 and 12 hours (t-test, $p < .011$). In young female rats (Fig. 2.17.2), LPS treatment produced a decrease in PCV between 1.5 and 3 hours (t-test, $p < .474$) and again at 48

hours paralleling and not different than that of the control animals (t-test, $p < .540$).

In middle-aged male rats (Fig. 2.18.1), LPS treatment produced a decrease in PCV between 1.5 and 12 hours ($p < .001$). In middle-aged female rats (Fig. 2.18.2), LPS treatment produced a decrease in PCV between 0.5 and 3 hours (t-test, $p < .001$), and again at hour 48 ($p < .054$).

In old male rats (Fig. 2.19.1), LPS treatment produced a decrease in PCV at hour 1.5 and 3, and again at hour 24 and 48 hours. In old female rats (Fig. 3.18.2), LPS treatment produced a decrease in PCV between 1.5 and 3 hours ($p < .02$).

Except for the generally higher PCV in the male there were no gender differences in the PCV response to LPS.

Other measurements that are not significantly affected by LPS treatment are shown in Tables 2.1.1- 2.5.6

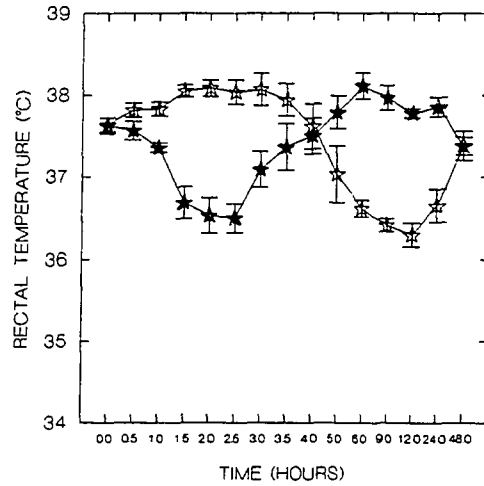


Figure 2.2.1. The effect of treatment on the rectal temperature (T_R) of young male rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\star , $n=6$), LPS (\star , $n=9$). The independent variables age, gender and treatment interact ($p < .014$).

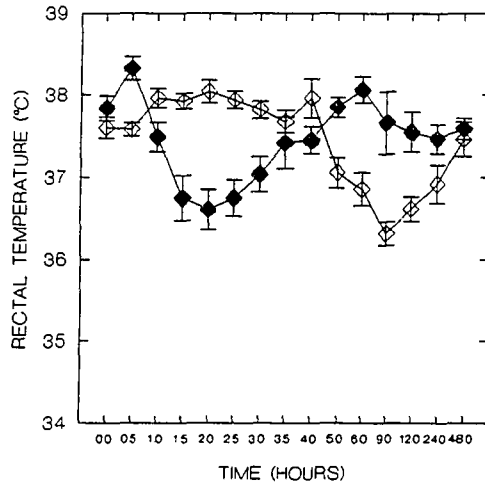


Figure 2.2.2. The effect of treatment on the T_R of young female rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). The independent variables age, gender and treatment interact ($p < .014$).

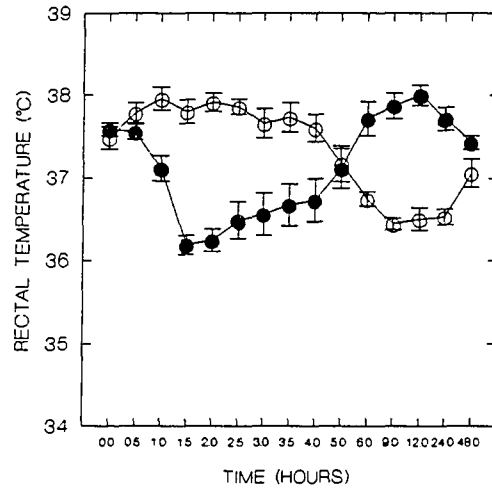


Figure 2.3.1. The effect of treatment on the T_R of middle-aged male rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$).

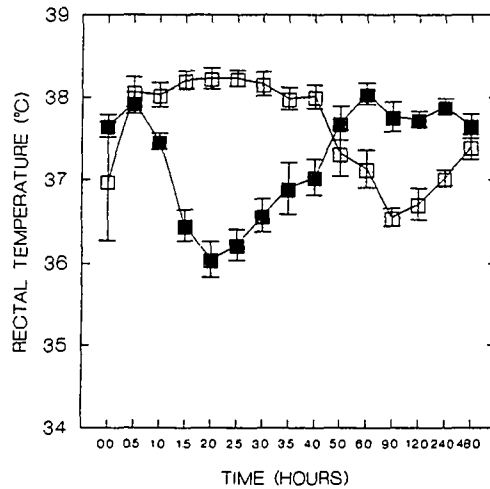


Figure 2.3.2. The effect of treatment on the T_R of middle-aged female rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$). The independent variables age, gender and treatment interact ($p < .014$).

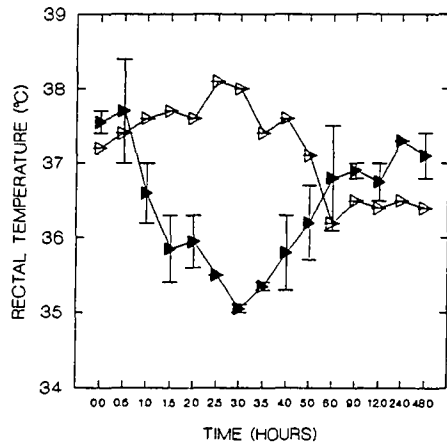


Figure 2.4.1. The effect of treatment on the T_R of old male rats. Saline (\triangleright , n=1), LPS (\blacktriangleright , n=2).

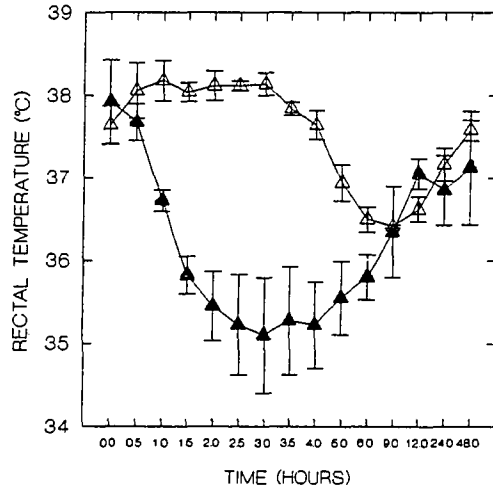


Figure 2.4.2. The effect of treatment on the T_R of old female rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is an interaction between the independent variables age, gender and treatment ($p < .014$).

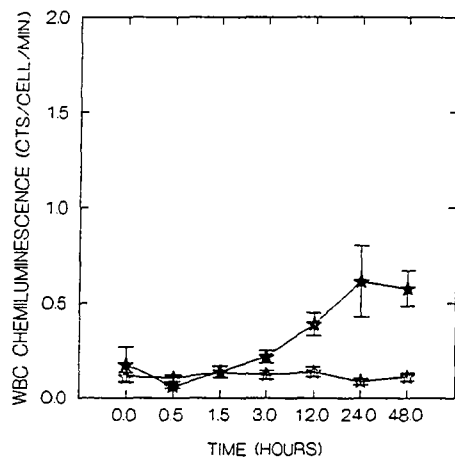


Figure 2.5.1. The effect of treatment on the WBC chemiluminescence (CL) in young male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .002$). Saline (\star , $n=6$), LPS (\star , $n=9$). Gender and treatment ($p < .012$), and age and treatment ($p < .012$) interact, but not age, gender and treatment ($p < .079$).

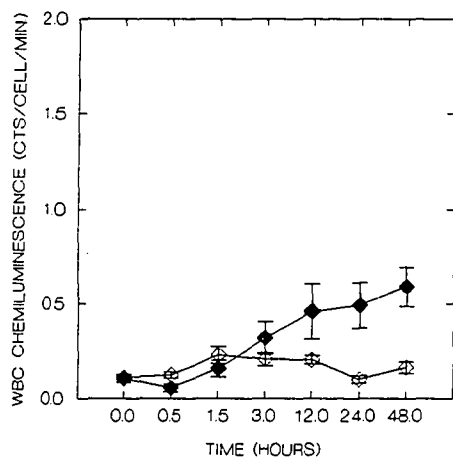


Figure 2.5.2. The effect of treatment on the WBC CL in young female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .005$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). There is an interaction between gender and treatment ($p < .012$), and age and treatment ($p < .012$), but not between age, gender and treatment ($p < .079$).

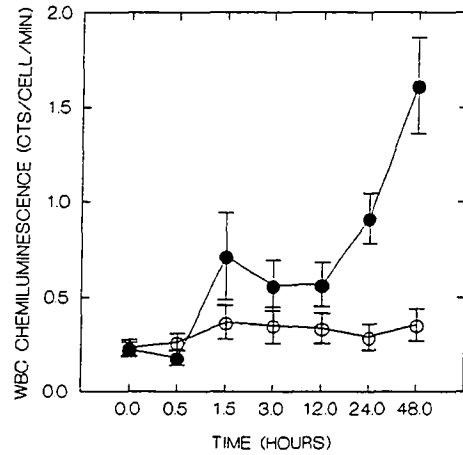


Figure 2.6.1. The effect of treatment on the WBC CL in middle-aged male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .002$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$). There is an interaction between gender and treatment ($p < .012$), and age and treatment ($p < .012$), but not between age, gender and treatment ($p < .079$).

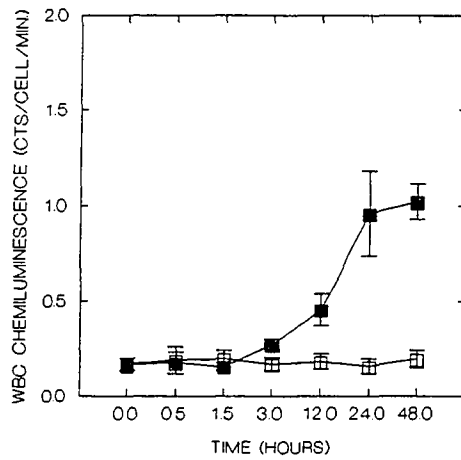


Figure 2.6.2. The effect of treatment on the WBC CL in middle-aged female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$). There is an interaction between gender and treatment ($p < .012$), and age and treatment ($p < .012$), but not between age, gender and treatment ($p < .079$).

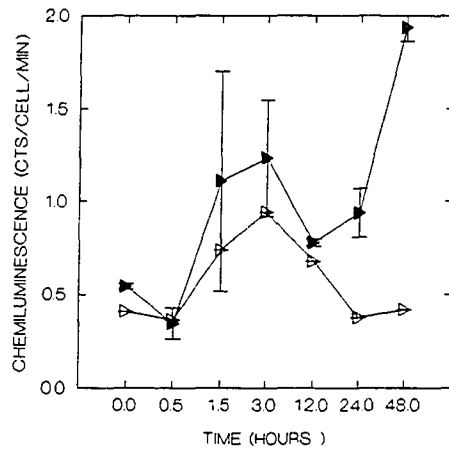


Figure 2.7.1. The effect of treatment on the WBC CL in old male rats during the acute phase of the protocol. Saline (∇ , n=2), LPS (\blacktriangleright , n=1).

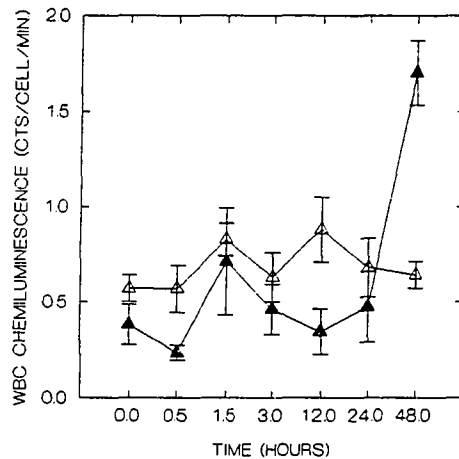


Figure 2.7.2. The effect of treatment on the WBC CL in old female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .004$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is an interaction between gender and treatment ($p < .012$), and age and treatment ($p < .012$), but not between age, gender and treatment ($p < .079$).

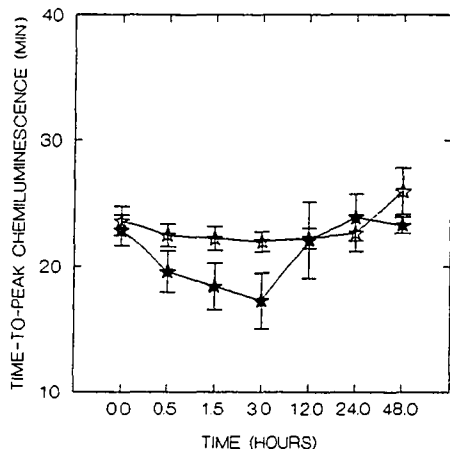


Figure 2.8.1. The effect of treatment on the time-to-peak (TTP) CL in young male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\star , $n=6$), LPS (\star , $n=9$). There is an interaction between gender and treatment ($p < .001$), but not between age, gender and treatment ($p < .916$).

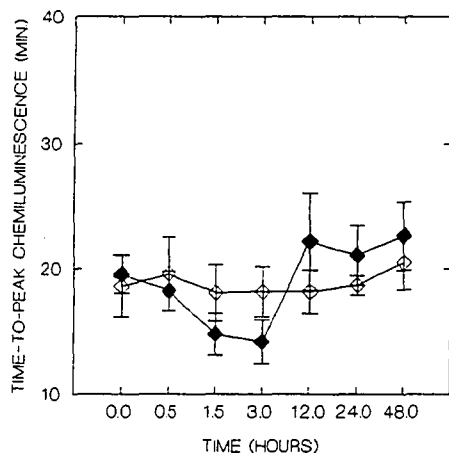


Figure 2.8.2. The effect of treatment on the TTP CL in young female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .165$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). Gender and treatment interact ($p < .001$), but age, gender and treatment do not ($p < .916$).

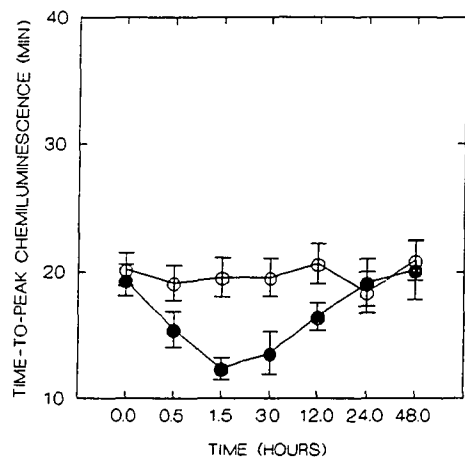


Figure 2.9.1. The effect of treatment on the TTP CL in middle-aged male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$). Gender and treatment interact ($p < .001$), but age, gender and treatment do not ($p < .916$).

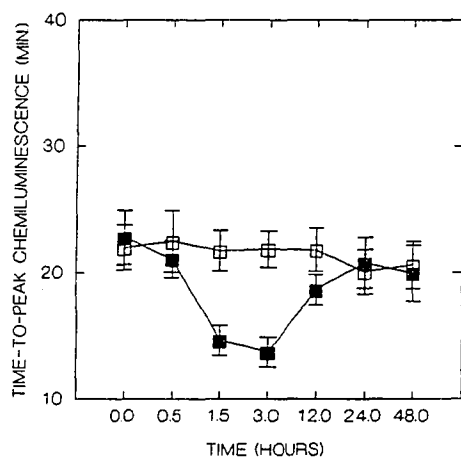


Figure 2.9.2. The effect of treatment on the TTP CL in middle-aged female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$). Gender and treatment interact ($p < .001$), but age, gender and treatment do not ($p < .916$).

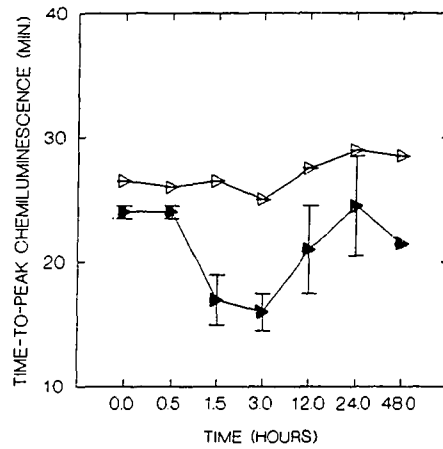


Figure 2.10.1. The effect of treatment on the TTP CL in old male rats during the acute phase of the protocol. Saline (∇ , n=1), LPS (\blacktriangleright , n=2).

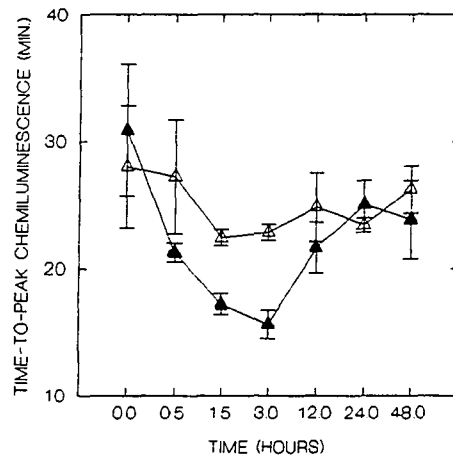


Figure 2.10.2. The effect of treatment on the TTP CL in old female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .003$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is an interaction between gender and treatment ($p < .001$), but not between age, gender and treatment ($p < .916$).

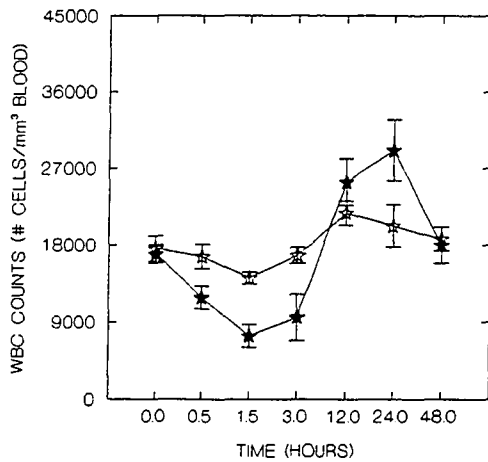


Figure 2.11.1. The effect of treatment on the WBC counts of young male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test $p < .001$). Saline (\star , $n=6$), LPS (\star , $n=9$). There is no interaction between the independent variables age, gender and treatment ($p < .833$).

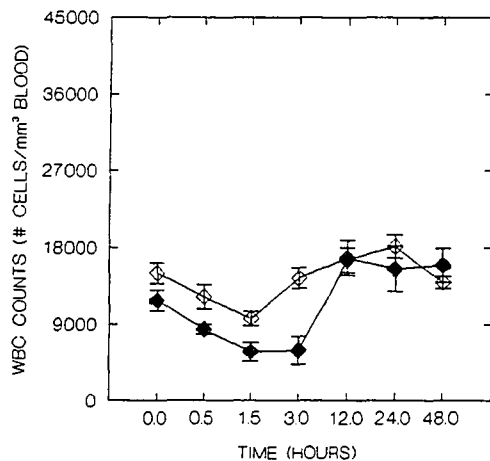


Figure 2.11.2. The effect of treatment on the WBC counts of young female rats during the acute phase of the protocol (MEAN \pm S.E.M.; t-test, $p < .002$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). There is no interaction between the independent variables age, gender and treatment ($p < .833$).

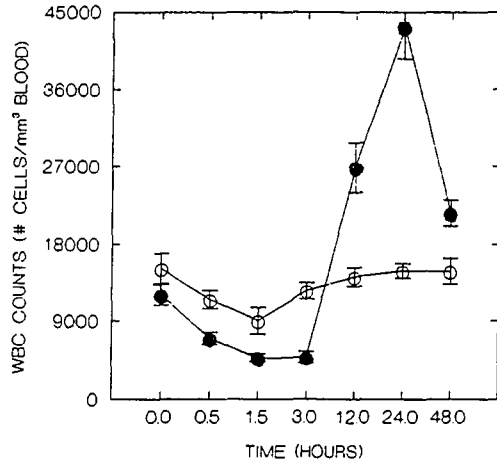


Figure 2.12.1. The effect of treatment on the WBC count of middle-aged male rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (O, $n=7$), LPS (●, $n=7$). There is no interaction between the independent variables age, gender and treatment ($p < .833$).

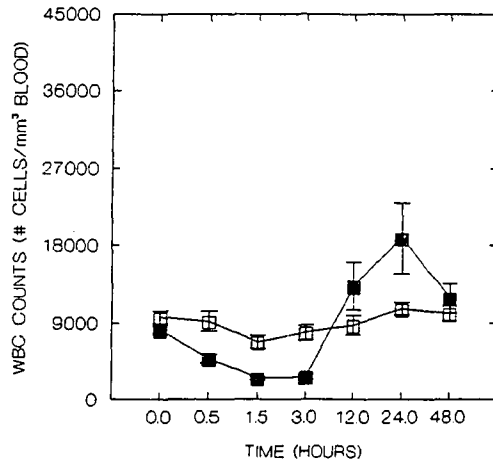


Figure 2.12.2. The effect of treatment of the WBC counts of middle-aged female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .001$). Saline (□, $n=9$), LPS (■, $n=9$). There is no interaction between the independent variables age, gender and treatment ($p < .833$).

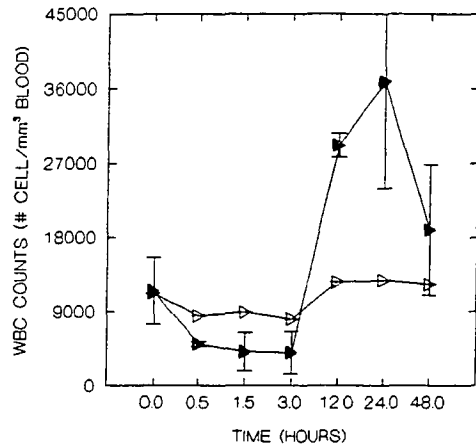


Figure 2.13.1. The effects of treatment on old male rats WBC counts during the acute phase of the treatment. Saline (∇ , n=1), LPS (\blacktriangleright , n=2,).

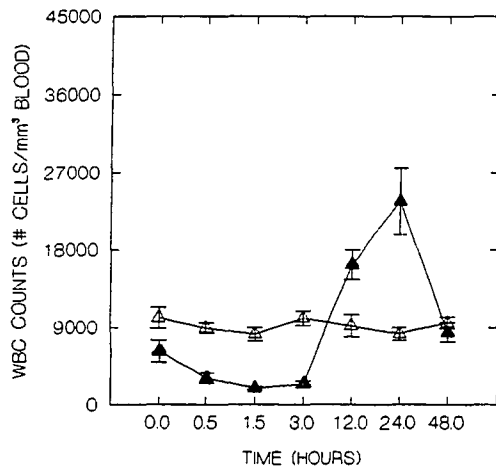


Figure 2.13.2. The effects of treatment on old female rats WBC counts during the acute phase of the treatment (MEAN \pm S.E.M., t-test, $p < .001$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is no interaction between age, gender and treatment ($p < .833$).

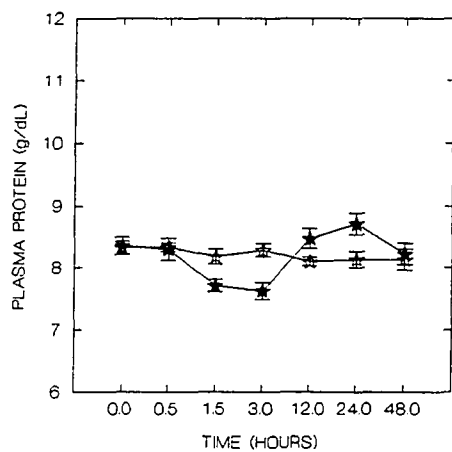


Figure 2.14.1. The effect of treatment on the plasma protein concentration of young male rats (MEAN \pm S.E.M.; t-test, $p < .002$). Saline (\star , $n=6$), LPS (\blackstar , $n=9$). There is no interaction between the independent variables age, gender, and treatment ($p < .846$).

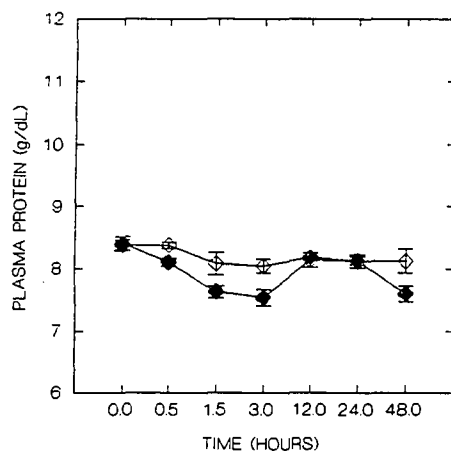


Figure 2.14.2. The effect of treatment on the plasma protein concentration of young female rats (MEAN \pm S.E.M., t-test, $p < .006$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). There is no interaction between the independent variables age, gender, and treatment ($p < .846$).

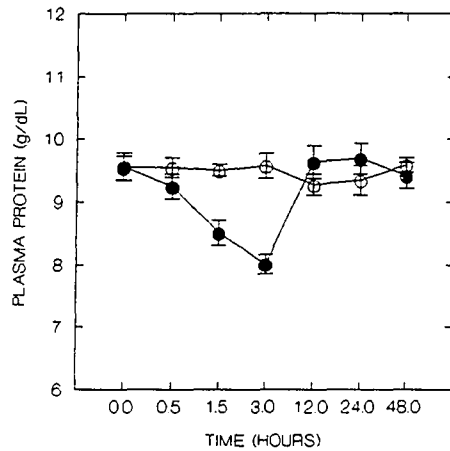


Figure 2.15.1. The effect of treatment on the plasma protein concentration of middle-aged male rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$). There is no interaction between the independent variables age, gender, and treatment ($p < .846$).

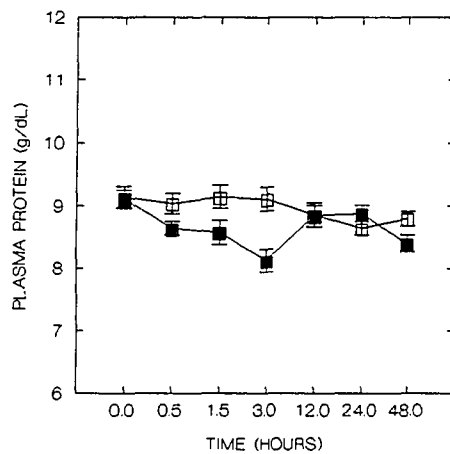


Figure 2.15.2. The effect of treatment on the plasma protein concentration of middle-aged female rats during the acute phase of the protocol (MEAN \pm S.E.M.; t-test, $p < .002$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$). There is no interaction between the independent variables age, gender, and treatment ($p < .846$).

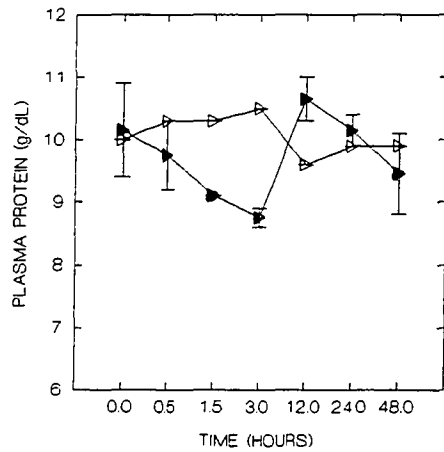


Figure 2.16.1. The effect of treatment on the plasma protein concentration of old male rats during the acute phase of the protocol. Saline (Δ , n=1), LPS (\blacktriangleright , n=2).

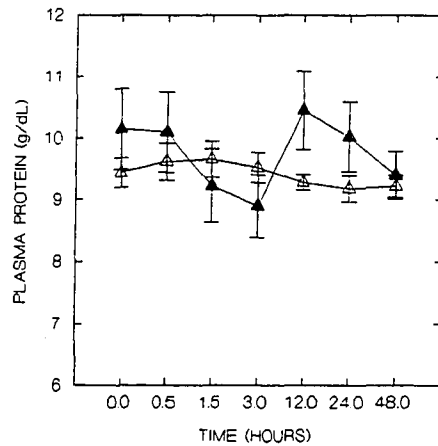


Figure 2.16.2. The effect of treatment on the plasma protein concentration of old female rats during the acute phase of the protocol (MEAN \pm S.E.M., t-test, $p < .161$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is no interaction between the independent variables age, gender, and treatment ($p < .846$).

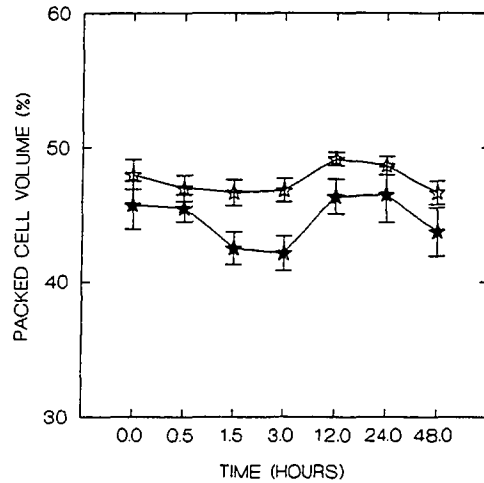


Figure 2.17.1. The effect of treatment on the packed cell volume of young male rats (MEAN \pm S.E.M., t-test, $p < .011$). Saline (\star , $n=6$), LPS (\star , $n=9$). There is no interaction between the independent variables ($p < .439$).

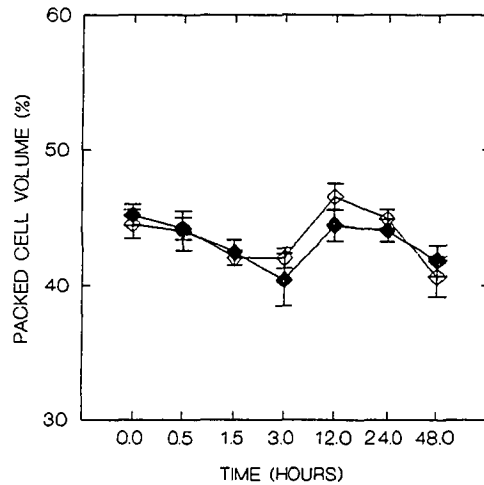


Figure 2.17.2. The effect of treatment on the packed cell volume of young female rats (MEAN \pm S.E.M., t-test, $p < .201$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). There is no interaction between the independent variables ($p < .439$).

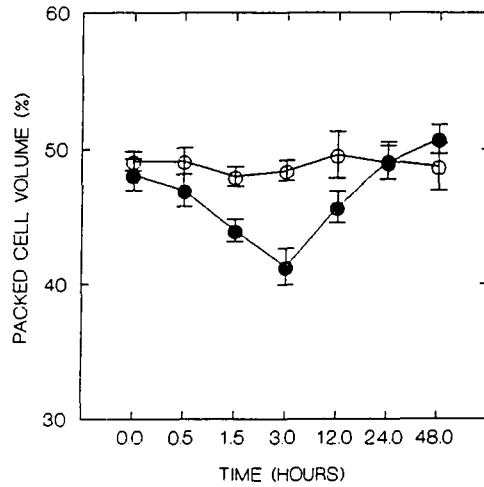


Figure 2.18.1. The effect of treatment on the packed cell volume of middle-aged male rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (O, $n=7$), LPS (●, $n=7$). There is no interaction between the independent variables ($p < .439$).

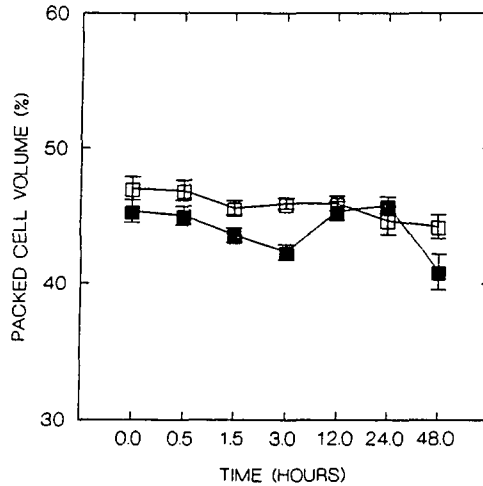


Figure 2.18.2. The effect of treatment on the packed cell volume of middle-aged female rats (MEAN \pm S.E.M., t-test, $p < .001$). Saline (□, $n=9$), LPS (■, $n=9$). There is no interaction between the independent variables ($p < .439$).

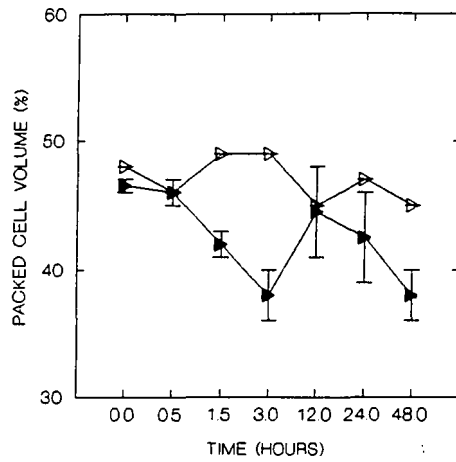


Figure 2.19.1. The effect of treatment on the packed cell volume of old male rats during the acute phase of the protocol. Saline (Δ , n=1), LPS (\blacktriangleright , n=2).

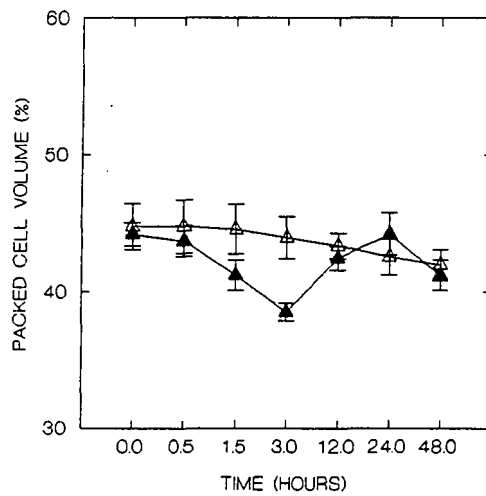


Figure 2.19.2. The effect of treatment on the packed cell volume of old female rats (MEAN + S.E.M., $p < .036$; t-test, $p < .020$). Saline (Δ , n=5), LPS (\blacktriangle , n=4). There is no interaction between the independent variables ($p < .439$).

II. LPS treatment: chronic phase

A. Body Temperature

In young female rats (Fig. 2.20.1 and 2.20.2), rectal temperature tended to be higher than the control rats through days 4-22 (ANOVA, $p < .025$) achieving statistical significance on day 20 (t-test, $p < .046$). In contrast, the middle-aged male rats' (Fig. 2.21.1 and 2.21.2) rectal temperature following LPS treatment was the same as that of the control rats through day 12, but tended to be decreased through day 24 (ANOVA, $p < .005$) achieving statistical significance on days 16 (t-test, $p < .006$) and 18 (t-test, $p < .036$). In old female rats' (Fig. 2.22.1 and 2.22.2), rectal temperature tended to be lower following LPS treatment through day 19 and then became higher than that of the control rats between day 20 and 23 (ANOVA, $p < .025$), although not statistically significant by t-test.

B. WBC Mobility

In young male rats (Fig. 2.23), WBC mobility was lower in LPS treated rats between days 6 and 20 (ANOVA, $p < .002$) and statistically significant on day 20 (t-test, $p < .026$). In middle-aged female rats (Fig. 2.24), WBC mobility was lower in LPS treated rats between days 13 and 24 (ANOVA, $p < .017$) and statistically significant on day 20 (t-test, $p < .019$).

C. Plasma Protein

In young male rats (Fig. 2.24), the plasma protein concentration was higher following LPS treatment between days 13 and 24 (ANOVA, $p < .003$) and statistically significant on day 20 (t-test, $p < .05$).

D. Erythrocyte Sedimentation Rate

In young male rats (Fig. 2.25), erythrocyte sedimentation rate (ESR) was higher following LPS treatment between days 6 and 24 (ANOVA, $p < .017$), but not statistically significant by t-test.

E. Packed Cell Volume

In young male rats (Fig. 2.28), packed cell volume was lower in LPS treated rats on days 6 and 13 (ANOVA, $p < .021$) achieving statistical significance on day 6 (t-test, $p < .03$). In middle-aged male rats (Fig. 2.29), packed cell volume was significantly lower in LPS treated rats (ANOVA, $p < .002$) between days 6 (t-test, $p < .004$) and 13 (t-test, $p < .016$). In middle-aged female rats (Fig. 2.30), packed cell volume was lower in LPS treated rats at day 6 (ANOVA, $p < .003$; t-test, $p < .016$). In old male rats (Fig. 2.31), packed cell volume appears to be lower in LPS treated rats between days 6 and 24.

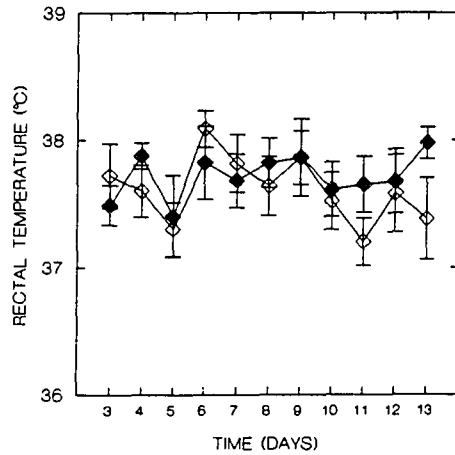


Figure 2.20.1. The effect of LPS (0.5 mg/kg, I.P.) treatment on the rectal temperature (T_R) of young female rats on days 3 through 13 (chronic) (MEAN \pm S.E.M., ANOVA $p < .025$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$).

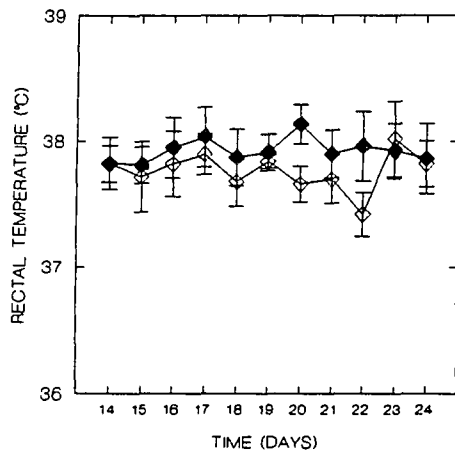


Figure 2.20.2. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic T_R of young female rats on days 14 through 24 (Mean \pm S.E.M., ANOVA $p < .025$). Saline (\diamond , $n=5$), LPS (\blacklozenge , $n=8$). Age and gender ($p < .048$), gender and treatment ($p < .047$), age and treatment ($p < .002$) and age, gender and treatment interact ($p < .035$).

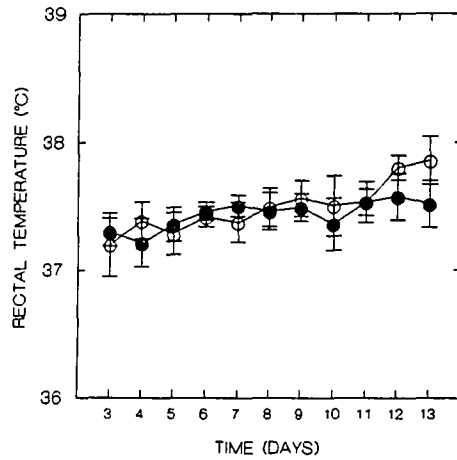


Figure 2.21.1. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic T_R of middle-aged male rats on days 3 through 13 (Mean \pm S.E.M., ANOVA $p < .005$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$).

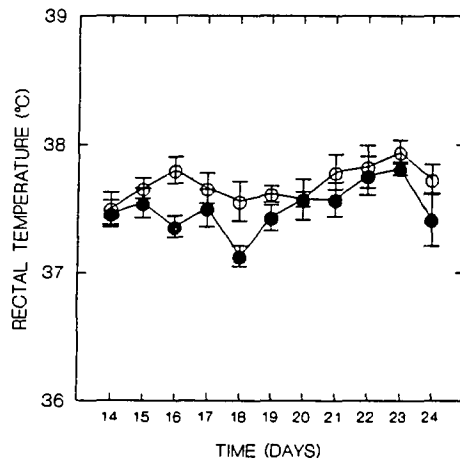


Figure 2.21.2. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic T_R of middle-aged male rats on days 14 through 24 (MEAN \pm S.E.M., ANOVA $p < .005$). Saline (\circ , $n=7$), LPS (\bullet , $n=7$). Age and gender, ($p < .048$) gender and treatment ($p < .047$), age and treatment ($p < .002$) and age, gender and treatment interact ($p < .035$).

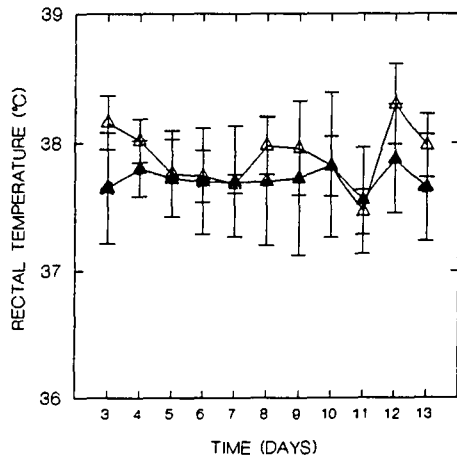


Figure 2.22.1. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic T_R of old female rats on days 3 through 13 (Mean \pm S.E.M., ANOVA $p < .025$). Saline (Δ , $n=5$), LPS (\blacktriangle , $n=4$).

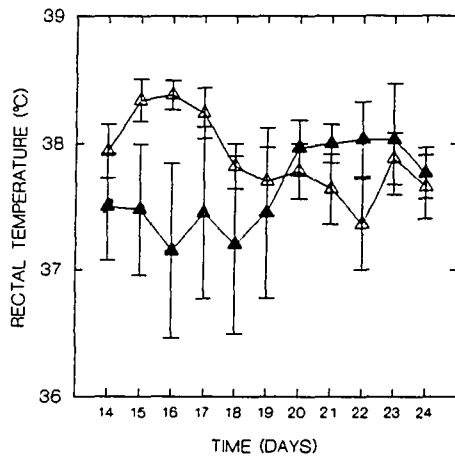


Figure 2.22.2. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic T_R of old female rats on days 14 through 24 (Mean \pm S.E.M., ANOVA $p < .025$). Saline (Δ , $n=5$), LPS (\blacktriangle , $n=4$). Age and gender, ($p < .048$) gender and treatment ($p < .047$), age and treatment ($p < .002$) and age, gender and treatment interact ($p < .035$).

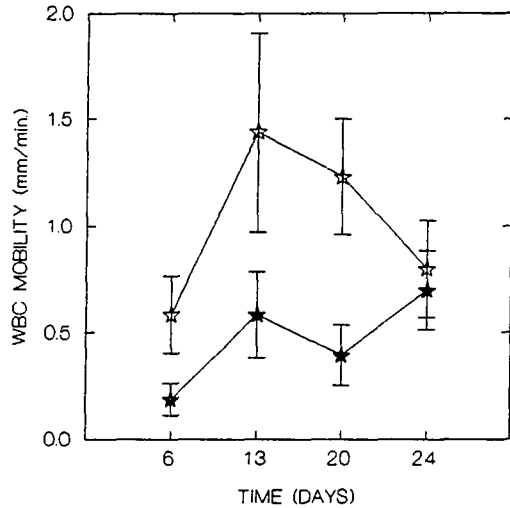


Figure 2.23. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic WBC mobility of young male rats (Mean \pm S.E.M., ANOVA $p < .002$). Saline (\star , $n=6$), LPS (\star , $n=9$).

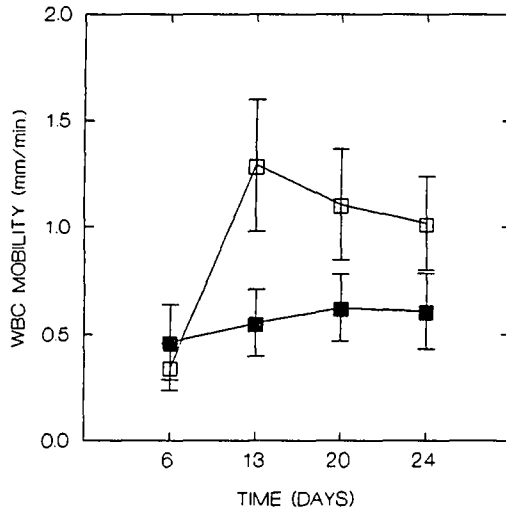


Figure 2.24. The effect of LPS (0.5 mg/kg, I.P.) on the chronic WBC mobility of middle-aged female rats (Mean \pm S.E.M., ANOVA $p < .017$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$).

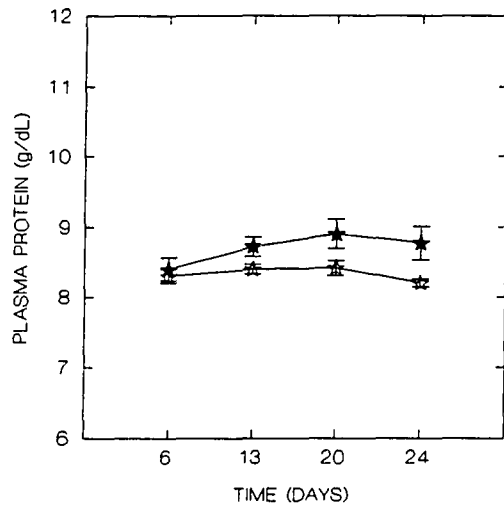


Figure 2.25. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic plasma protein concentration of young male rats (MEAN \pm S.E.M., ANOVA $p < .003$). Saline (☆, $n=6$), LPS (★, $n=9$).

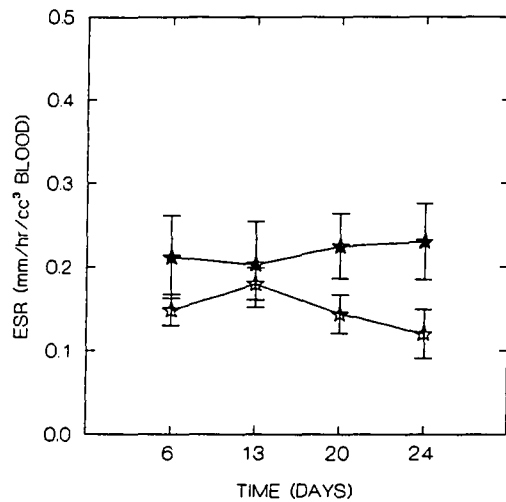


Figure 2.26. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic erythrocyte sedimentation rate (ESR) of young male rats (MEAN \pm S.E.M., ANOVA $p < .017$). Saline (\star , $n=6$), LPS (\blackstar , $n=9$).

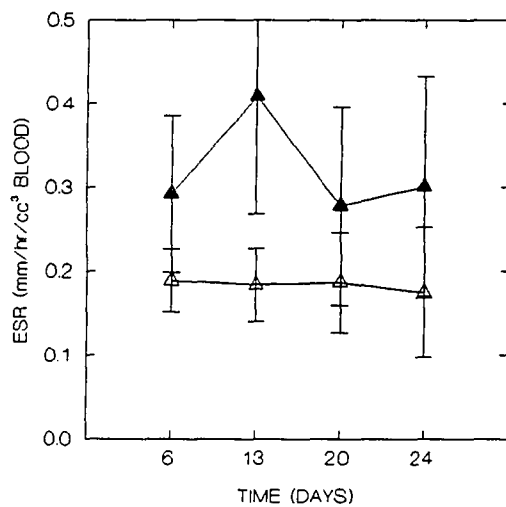


Figure 2.27. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic ESR of old female rats (MEAN \pm S.E.M., ANOVA $p < .017$). Saline (Δ , $n=5$), LPS (\blacktriangle , $n=4$).

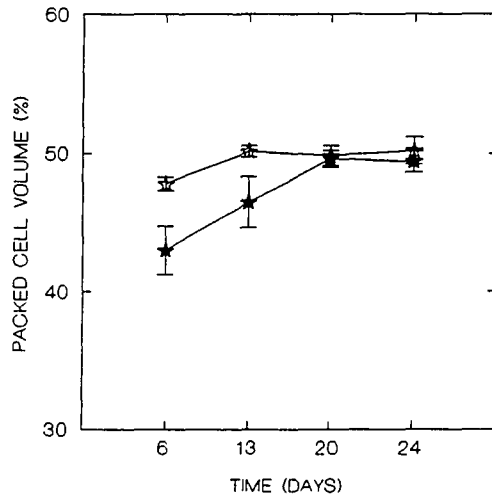


Figure 2.28. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic packed cell volume (PCV) of young male rats (MEAN \pm S.E.M., ANOVA $p < .021$). Saline (\star , $n=6$), LPS (\star , $n=9$).

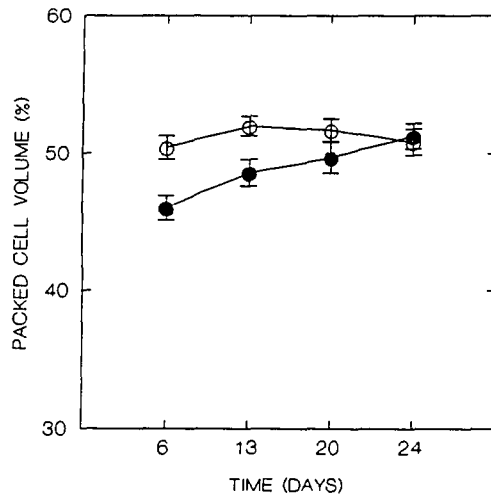


Figure 2.29. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic PCV of middle-aged male rats (MEAN \pm S.E.M., ANOVA $p < .002$). Saline (O, $n=7$), LPS (●, $n=7$).

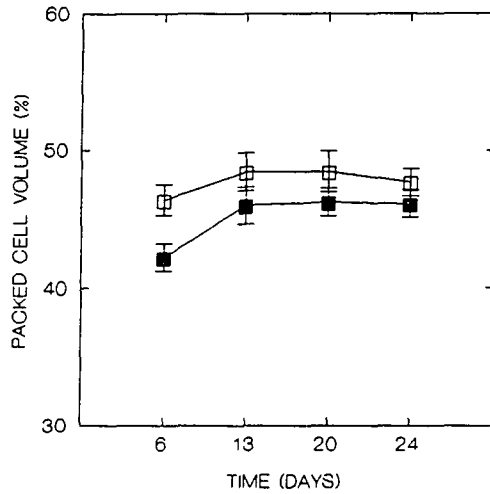


Figure 2.30. The effect of LPS (0.5 mg/kg, I.P.) treatment on the chronic PCV of middle-aged female rats (MEAN \pm S.E.M., ANOVA $p < .003$). Saline (\square , $n=9$), LPS (\blacksquare , $n=9$).

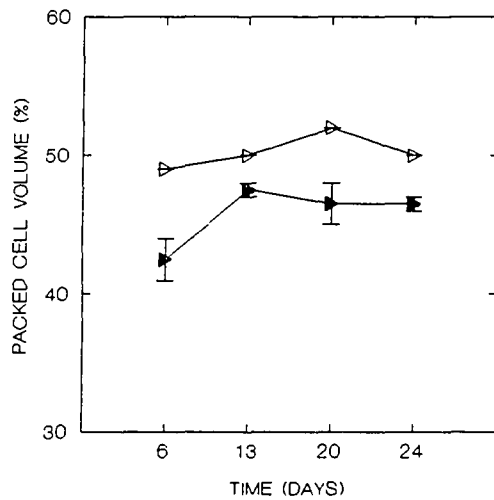


Figure 2.31. The effect of LPS (0.5 mg/kg, I.P.) treatment on the PCV of old male rats on days 6 through 24 (MEAN \pm S.E.M., ANOVA $p < .006$). Saline (\triangleright , $n=1$), LPS (\blacktriangleright , $n=2$).

TABLE 2.1.1. YOUNG MALE ACUTE BODY WEIGHT

VARIABLE	TIME HOUR	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT								
ACUTE	0	323.4	24.2	6	378.6	32.6	9	.196
	0.5	323.1	23.9	6	376.3	32.2	9	.208
	1.0	322.7	24.0	6	374.6	32.7	9	.223
	1.5	321.2	24.3	6	372.3	32.6	9	.231
	2.0	319.4	24.2	6	370.9	32.8	9	.229
	2.5	317.4	24.3	6	356.1	33.9	8	.372
	3.0	315.7	24.4	6	367.6	32.8	9	.227
	3.5	315.3	23.3	5	379.6	34.3	9	.250
	4.0	313.6	24.7	6	364.8	32.5	9	.232
	5.0	312.8	24.8	6	362.6	32.5	9	.241
	6.0	311.3	24.9	6	360.9	32.5	9	.246
	9.0	309.2	24.5	6	357.3	31.9	9	.253
	12.0	306.4	24.5	6	355.1	31.7	9	.246
	24.0	295.6	23.3	6	342.5	31.4	9	.251
	48.0	319.9	22.3	6	359.6	31.4	9	.321

TABLE 2.1.2. YOUNG MALE CHRONIC BODY WEIGHT

VARIABLE	TIME DAY	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT CHRONIC	3	322.8	22.3	6	366.8	30.9	9	.269
	4	326.0	21.7	6	368.4	30.8	9	.282
	5	328.7	21.1	6	373.2	30.6	9	.252
	6	331.3	21.7	6	375.3	30.3	9	.259
	7	333.4	21.8	6	377.3	29.7	9	.256
	8	334.8	20.7	6	378.6	29.1	9	.241
	9	337.4	20.4	6	380.8	28.9	9	.242
	10	338.7	21.1	6	385.8	28.4	9	.206
	11	342.8	20.2	6	386.5	28.3	9	.230
	12	342.7	21.5	6	390.7	27.7	9	.194
	13	347.5	20.9	6	392.9	27.8	9	.214
	14	347.8	20.5	6	395.6	27.8	9	.190
	15	353.6	20.2	6	396.3	27.4	9	.232
	16	355.4	20.2	6	398.4	27.3	9	.228
	17	355.5	21.0	6	399.6	27.7	9	.226
	18	357.0	20.6	6	402.5	28.5	9	.219
	19	357.8	20.2	6	405.0	28.0	9	.196
	20	359.4	20.3	6	404.1	27.2	9	.211
	21	360.3	20.7	6	406.6	27.3	9	.199
	22	362.6	20.6	6	405.3	25.9	9	.219
	23	361.3	20.6	6	407.8	27.7	9	.201
	24	362.0	20.9	6	403.4	30.1	8	.211

TABLE 2.1.3. YOUNG MALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
PCV	HOUR							p value t-test
ACUTE	0.0	48%	1.0	6	46%	1.8	8	.266
	0.5	47%	1.0	6	46%	1.0	8	.306
	1.5	47%	1.0	6	43%	1.2	8	.020
	3.0	47%	0.9	6	42%	1.3	8	.011
	12.0	49%	0.5	6	46%	1.3	8	.079
	24.0	49%	0.7	6	47%	2.1	8	.349
	48.0	47%	0.9	6	44%	1.8	8	.178
CHRONIC								
	DAY							
	6	48%	0.5	6	43%	1.8	8	.030
	13	50%	0.4	6	47%	1.8	8	.089
	20	50%	0.7	6	50%	0.6	8	.825
	24	50%	1.0	5	50%	0.8	8	.546

TABLE 2.1.4. YOUNG MALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
ESR	HOURL							
ACUTE	0.0	.137	.023	6	.130	.020	8	.807
	0.5	.246	.024	6	.201	.043	8	.384
	1.5	.228	.036	6	.165	.042	8	.278
	3.0	.155	.028	6	.193	.062	8	.590
	12.0	.154	.031	6	.173	.025	8	.645
	24.0	.091	.028	6	.227	.046	8	.028
	48.0	.146	.028	6	.236	.066	8	.237
CHRONIC								
	DAY							
	6	.149	.018	6	.212	.050	8	.261
	13	.180	.019	6	.203	.051	8	.682
	20	.144	.023	6	.225	.039	8	.099
	24	.120	.029	5	.230	.045	7	.070

TABLE 2.1.5. YOUNG MALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
WBC MOBILITY	HOURL							
ACUTE								
	0.0	.380	.144	6	.172	.078	8	.240
	0.5	.479	.189	6	.452	.211	8	.928
	1.5	.547	.267	6	.251	.140	8	.356
	3.0	.988	.257	6	.406	.306	8	.171
	12.0	1.043	.310	6	.250	.134	8	.052
	24.0	.875	.386	6	.158	.140	8	.129
	48.0	.754	.303	6	.416	.191	8	.371
CHRONIC								
	DAY							
	6	.583	.179	6	.188	.075	8	.082
	13	1.438	.467	6	.583	.200	8	.137
	20	1.230	.270	6	.395	.140	8	.026
	24	.796	.228	5	.697	.185	7	.744

TABLE 2.1.6. YOUNG MALE POST-MORTEM

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
% WATER	POST MORTEM							p value t-test
HEART		76.9	.226	6	77.0	.144	8	.804
LUNG		83.5	2.2	6	79.8	1.1	8	.177
LIVER		70.2	.277	6	68.5	.627	8	.032
PERFUSED LIVER		78.1	.424	6	79.8	1.1	6	.189
LIVER HOMOGENATE		98.1	.227	6	97.1	.966	6	.355
HOMOGENATE DRY WT.		.004	.001	5	.006	.003	5	.361
LIVER CL		19431300	13066500	5	21394700	26137300	5	.886
LIVER % BODY WEIGHT		3.5	-	1	3.4	-	1	-

TABLE 2.2.1. YOUNG FEMALE ACUTE BODY WEIGHT

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
BODY WEIGHT								
ACUTE	HOUR							
	0.0	211.6	14.2	5	212.3	9.7	8	.986
	0.5	210.6	13.5	5	210.6	9.3	8	.998
	1.0	209.0	13.3	5	209.1	9.5	8	.997
	1.5	208.2	13.4	5	209.0	9.7	8	.960
	2.0	206.2	13.3	5	207.0	9.5	8	.959
	2.5	205.5	13.3	5	205.8	9.4	8	.985
	3.0	204.5	12.3	5	204.6	9.2	8	.995
	3.5	204.4	16.5	4	202.1	10.8	6	.913
	4.0	201.9	13.0	5	202.3	9.1	8	.982
	5.0	201.5	13.0	5	201.2	9.0	8	.983
	6.0	201.0	13.0	5	199.9	9.0	8	.947
	9.0	199.6	13.2	5	198.2	8.9	8	.933
	12.0	197.8	13.1	5	196.7	8.9	8	.947
	24.0	191.6	13.3	5	189.0	8.0	8	.873
	48.0	210.3	14.2	5	203.3	8.9	8	.689

TABLE 2.2.2. YOUNG FEMALE CHRONIC BODY WEIGHT

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT								
CHRONIC	DAY							
	3	212.2	14.2	5	207.8	8.8	8	.800
	4	215.5	14.1	5	214.2	8.2	8	.938
	5	217.8	13.0	5	215.3	7.7	8	.876
	6	221.2	13.2	5	216.9	7.9	8	.789
	7	220.9	12.6	5	219.1	7.0	8	.903
	8	223.3	11.6	5	223.2	8.1	8	.996
	9	222.0	12.7	5	226.5	7.6	8	.771
	10	224.7	12.1	5	226.2	7.7	8	.923
	11	223.5	11.2	5	226.0	7.2	8	.854
	12	228.3	11.5	5	230.0	7.4	8	.902
	13	226.7	11.4	5	231.1	8.1	8	.764
	14	225.6	9.9	5	229.6	7.5	8	.760
	15	228.4	10.5	5	227.7	5.9	8	.956
	16	230.8	9.8	5	230.4	6.7	8	.969
	17	230.3	9.6	5	233.7	6.9	8	.780
	18	231.9	7.7	5	235.0	7.1	8	.779
	19	230.2	7.8	5	234.0	6.1	8	.707
	20	232.6	9.8	5	235.4	7.1	8	.822
	21	234.6	8.6	5	236.9	6.9	8	.839
	22	237.0	8.5	5	238.0	6.2	8	.927
	23	237.9	7.9	5	236.6	5.7	8	.893
	24	237.7	6.4	5	238.0	7.1	8	.976

TABLE 2.2.3. YOUNG FEMALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
PCV								
ACUTE	HOURLY							
	0.0	45%	1.0	4	45%	0.8	6	.628
	0.5	44%	1.5	4	44%	0.8	6	.925
	1.5	42%	0.9	5	42%	0.5	8	.720
	3.0	40%	1.9	5	42%	0.7	8	.474
	12.0	44%	1.2	5	47%	1.0	8	.201
	24.0	44%	0.8	5	45%	0.7	8	.441
	48.0	42%	1.1	5	41%	1.5	8	.540
CHRONIC	DAY							
	6	45	1.0	5	40%	1.8	8	.090
	13	46	1.3	5	45%	1.2	8	.536
	20	47	1.1	5	47%	1.0	8	.936
	24	48	1.4	5	47%	0.7	8	.599

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TABLE 2.2.4. YOUNG FEMALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
ESR								
ACUTE	HOURLY							
	0.0	.135	.045	4	.071	.016	6	.255
	0.5	.154	.040	4	.105	.010	6	.315
	1.5	.119	.019	4	.062	.019	6	.073
	3.0	.110	.023	4	.048	.018	6	.077
	12.0	.135	.011	4	.101	.023	6	.220
	24.0	.111	.030	4	.068	.022	6	.285
	48.0	.108	.016	4	.215	.038	6	.036
CHRONIC	DAY							
	6	.194	.032	4	.205	.029	6	
	13	.140	.020	4	.164	.031	6	
	20	.147	.024	4	.169	.016	6	
	24	.135	.049	4	.124	.029	6	

TABLE 2.2.5. YOUNG FEMALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
WBC MOBILITY								
ACUTE	HOURLY							
	0.0	1.003	.356	4	.334	.306	6	.157
	0.5	0.406	.094	4	.292	.251	6	.601
	1.5	0.570	.241	4	.104	.082	6	.148
	3.0	1.000	.368	4	.008	.066	6	.087
	12.0	.408	.213	4	.752	.304	6	.381
	24.0	.594	.257	4	.663	.306	6	.868
	48.0	.438	.295	4	.547	.251	6	.787
CHRONIC	DAY							
	6	.813	.213	4	.647	.189	6	.579
	13	.470	.281	4	.483	.113	6	.967
	20	.533	.078	4	.793	.251	6	.360
	24	.660	.141	4	.897	.093	6	.214

TABLE 2.2.6. YOUNG FEMALE POST-MORTEM

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
% WATER	POST MORTEM							p value t-test
HEART		76.5	.455	9	76.7	.139	9	.637
LUNG		80.4	.338	9	80.1	1.593	9	.778
LIVER		10.1	0	4	9.1	.527	4	.402
PERFUSED LIVER		79.4	.687	7	78.9	2.341	7	.853
LIVER HOMOGENATE		97.7	.441	7	97..5	.317	7	.819
HOMOGENATE DRY WT.		.005	0	7	.005	.001	7	.855
LIVER CL		47763600	5951086	7	24287400	13209700	7	.099
LIVER % BODY WEIGHT		3.5	0	7	3.2	.093	7	.455

TABLE 2.3.1. MIDDLE AGE MALE ACUTE BODY WEIGHT

VARIABLE	TIME HOUR	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT								
ACUTE	0	533.9	8.3	7	490.9	11.2	7	.011
	0.5	532.4	8.6	7	488.8	11.2	7	.010
	1.0	530.9	8.8	7	486.5	11.6	7	.011
	1.5	528.7	8.5	7	485.0	11.2	7	.010
	2.0	527.4	8.4	7	485.1	11.3	7	.012
	2.5	526.4	8.7	7	484.9	11.2	7	.013
	3.0	524.2	8.5	7	484.9	11.4	7	.018
	3.5	522.9	8.6	7	484.1	11.3	7	.019
	4.0	521.8	8.2	7	482.7	11.2	7	.016
	5.0	520.2	8.2	7	479.1	10.4	7	.010
	6.0	519.2	8.1	7	475.4	9.9	7	.055
	9.0	516.5	8.1	7	470.3	10.2	7	.044
	12.0	512.7	8.0	7	466.9	10.5	7	.005
	24.0	506.7	8.5	7	455.0	10.9	7	.003
	48.0	525.9	7.0	7	464.2	12.1	7	.001

TABLE 2.3.2. MIDDLE AGE MALE CHRONIC BODY WEIGHT

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
BODY WEIGHT	DAY							p value t-test
CHRONIC	3	524.0	7.2	7	469.8	11.7	7	.003
	4	527.0	7.7	7	474.0	12.4	7	.005
	5	524.6	7.1	7	473.7	12.0	7	.005
	6	527.0	7.4	7	473.7	12.6	7	.005
	7	526.9	7.9	7	475.2	13.1	7	.007
	8	525.7	9.8	7	475.3	13.0	7	.010
	9	529.1	7.0	7	478.3	13.0	7	.007
	10	528.2	7.1	7	478.1	13.6	7	.010
	11	5289.0	8.0	7	480.5	13.3	7	.011
	12	527.4	7.8	7	479.2	13.6	7	.013
	13	526.6	8.1	7	481.9	12.6	7	.013
	14	529.0	7.8	7	483.6	12.2	7	.010
	15	529.0	7.8	7	483.5	12.6	7	.012
	16	526.8	7.4	7	487.6	11.7	7	.018
	17	525.6	7.3	7	484.8	11.7	7	.015
	18	527.4	8.3	7	488.6	11.7	7	.021
	19	541.8	18.6	7	488.2	11.8	7	.035
	20	540.0	19.4	7	486.9	11.3	7	.041
	21	540.9	18.2	7	488.5	12.0	7	.036
	22	529.7	7.7	7	489.3	12.1	7	.018
	23	529.2	6.9	7	491.3	12.2	7	.024
	24	531.3	7.1	7	491.1	10.8	7	.011

TABLE 2.3.3. MIDDLE AGE MALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
	HOUR							p value
PCV								t-test
ACUTE	0.0	49%	0.7	7	48%	1.2	7	.479
	0.5	49%	1.0	7	42%	3.8	7	.135
	1.5	48%	0.7	7	44%	0.8	7	.044
	3.0	48%	0.8	7	41%	1.3	7	.011
	12.0	48%	0.9	7	46%	1.2	7	.132
	24.0	49%	1.3	7	49%	1.4	7	1.000
	48.0	51%	1.0	7	49%	1.7	7	.342
CHRONIC								
	DAY							
	6	50%	0.9	7	46%	0.9	7	.004
	13	52%	0.7	7	49%	1.0	7	.016
	20	52%	0.8	7	50%	1.1	7	.187
	24	51%	1.0	7	51%	0.9	7	.756

TABLE 2.3.4. MIDDLE AGE MALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
ESR	HOURL							p value
								t-test
ACUTE	0.0	.158	.053	7	.074	.016	7	.175
	0.5	.348	.043	7	.230	.051	7	.100
	1.5	.174	.048	7	.123	.031	7	.390
	3.0	.231	.072	7	.155	.055	7	.418
	12.0	.208	.057	7	.164	.052	7	.587
	24.0	.292	.062	7	.140	.061	7	.104
	48.0	.332	.062	7	.218	.073	7	.259
CHRONIC								
	DAY							
	6	.300	.056	7	.363	.050	7	.420
	13	.346	.059	7	.392	.056	7	.580
	20	.325	.055	7	.299	.065	7	.766
	24	.337	.063	7	.358	.053	7	.802

TABLE 2.3.5. MIDDLE AGE MALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
	HOUR							p value
WBC								t-test
MOBILITY								
ACUTE								
	0.0	.121	.087	7	.107	.074	7	.903
	0.5	.393	.277	7	.108	.058	7	.350
	1.5	.143	.092	7	.075	.057	7	.545
	3.0	.143	.107	7	.299	.108	7	.584
	12.0	.407	.096	7	.036	.036	7	.007
	24.0	.304	.128	7	.089	.071	7	.173
	48.0	.304	.159	7	.125	.106	7	.368
CHRONIC								
	DAY							
	6	.699	.219	7	.322	.204	7	.233
	13	.501	.112	7	.626	.125	7	.474
	20	.321	.152	7	.518	.111	7	.318
	24	.501	.213	7	.750	.191	7	.401

TABLE 2.3.6. MIDDLE AGE MALE POST-MORTEM

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
% WATER	POST MORTEM							
HEART		76.5	1.744	7	77.1	.470	7	.254
LUNG		77.8	1.596	7	79.5	.740	7	.390
LIVER		68.2	.460	7	67.9	.710	7	.773
PERFUSED LIVER		76.4	1.440	7	77.6	1.085	7	.511
LIVER HOMOGENATE		96.67	.585	7	97.3	.724	7	.521
HOMOGENATE DRY WT.		.007	.001	7	.006	.001	7	.426
LIVER CL		25904200	14327500	7	26909000	16123200	7	.904
LIVER % BODY WEIGHT		4.0	.259	7	3.6	.175	7	.975

TABLE 2.4.1. MIDDLE AGE FEMALE ACUTE BODY WEIGHT

VARIABLE	TIME HOUR	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT	0	290.2	9.4	9	292.7	6.9	9	.832
ACUTE	0.5	288.5	9.9	9	291.8	7.1	9	.785
	1.0	287.2	9.9	9	290.4	7.2	9	.793
	1.5	286.2	9.6	9	289.6	7.1	9	.780
	2.0	285.2	9.6	9	289.2	6.8	9	.736
	2.5	284.2	9.4	9	292.0	7.0	9	.521
	3.0	283.4	9.4	9	289.0	6.9	9	.635
	3.5	274.0	9.4	9	286.1	7.1	9	.326
	4.0	281.5	9.2	9	287.5	6.8	9	.605
	5.0	280.5	9.1	9	285.8	6.8	9	.652
	6.0	279.9	9.0	9	283.8	6.7	9	.731
	9.0	278.4	8.8	9	280.9	6.6	9	.829
	12.0	276.1	8.9	9	268.1	11.6	9	.590
	24.0	268.7	9.4	9	272.6	7.2	9	.747
	48.0	288.5	10.4	9	287.0	6.5	9	.908

TABLE 2.4.2 MIDDLE AGE FEMALE CHRONIC BODY WEIGHT

VARIABLE	TIME DAY	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
BODY WEIGHT								
CHRONIC	3	287.1	10.2	9	290.8	7.2	9	.770
	4	287.8	9.7	9	291.4	6.7	9	.760
	5	288.5	9.6	9	291.4	6.6	9	.808
	6	287.8	9.7	9	290.5	7.0	9	.824
	7	286.0	9.7	9	290.4	6.5	9	.712
	8	288.3	10.1	9	292.0	6.5	9	.761
	9	287.7	9.5	9	293.4	6.6	9	.632
	10	288.8	9.3	9	293.6	7.1	9	.685
	11	287.4	9.3	9	296.0	7.0	9	.470
	12	287.2	9.1	9	297.3	7.5	9	.405
	13	286.5	9.2	9	294.9	7.6	9	.488
	14	287.2	9.9	9	298.6	7.4	9	.370
	15	288.0	9.8	9	296.6	7.1	9	.490
	16	289.9	9.7	9	298.8	7.7	9	.480
	17	288.9	9.3	9	297.7	6.4	9	.444
	18	289.2	9.2	9	297.3	8.0	9	.517
	19	289.0	9.1	9	296.2	6.8	9	.536
	20	288.4	9.4	9	296.1	6.9	9	.520
	21	288.9	9.5	9	296.7	6.5	9	.510
	22	287.3	9.0	9	295.0	6.4	9	.495
	23	290.1	9.2	9	296.2	6.2	9	.589
	24	289.1	9.4	9	295.9	6.2	9	.556

TABLE 2.4.3. MIDDLE AGE FEMALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
PCV	HOURLY							
ACUTE	0.0	47%	.8	9	45%	.8	9	.176
	0.5	47%	.7	9	45%	.7	9	.063
	1.5	46%	.5	9	44%	.5	9	.015
	3.0	46%	.4	9	42%	.5	9	.000
	12.0	46%	.5	9	45%	.7	9	.534
	24.0	45%	1.0	9	46%	.6	9	.375
	48.0	44%	.9	9	41%	1.3	9	.054
CHRONIC	DAY							
	6	46%	1.1	9	42%	1.0	9	.015
	13	48%	1.4	9	47%	2.2	9	.619
	20	48%	1.5	9	46%	1.0	9	.235
	24	48%	1.0	9	46%	1.0	9	.283

TABLE 2.4.4 MIDDLE AGE FEMALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
ESR	HOURLY							p value
								t-test
ACUTE	0.0	.060	.007	9	.067	.011	9	.615
	0.5	.149	.039	9	.103	.014	9	.301
	1.5	.065	.065	9	.047	.008	9	.277
	3.0	.070	.070	9	.097	.041	9	.549
	12.0	.062	.009	9	.076	.023	9	.580
	24.0	.028	.009	9	.116	.036	9	.052
	48.0	.086	.014	9	.298	.070	9	.023
CHRONIC								
	DAY							
	6	.135	.034	9	.197	.049	9	.318
	13	.162	.038	9	.112	.022	9	.280
	20	.161	.045	9	.089	.019	9	.182
	24	.113	.026	9	.067	.017	9	.165

TABLE 2.4.5 MIDDLE AGE FEMALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
WBC MOBILITY	HOURLY							
ACUTE								
	0.0	.143	.074	9	.197	.082	9	.632
	0.5	.143	.074	9	.054	.037	9	.311
	1.5	.036	.024	9	.071	.046	9	.516
	3.0	.586	.200	9	.251	.147	9	.204
	12.0	.071	.046	9	.071	.046	9	1.0
	24.0	.395	.162	9	.268	.169	9	.597
	48.0	.486	.110	9	.287	.118	9	.243
CHRONIC								
	DAY							
	6	.346	.111	9	.464	.176	9	.584
	13	1.290	.308	9	.555	.156	9	.063
	20	1.107	.261	9	.626	.157	9	.145
	24	1.019	.219	9	.609	.175	9	.171

TABLE 2.4.6. MIDDLE AGE FEMALE POST-MORTEM

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
% WATER	POST MORTEM							p value t-test
HEART		76.5	.2	9	76.0	.2	9	.637
LUNG		80.4	.9	9	80.1	.4	9	.778
LIVER		69.1	.6	7	65.3	3.6	7	.343
PERFUSED LIVER		79.4	1.9	7	78.9	1.7	7	.853
LIVER HOMOGENATE		97.7	.5	7	97.5	.5	7	.819
HOMOGENATE DRY WT.		.005	.001	7	.005	.001	7	.855
LIVER CL/DRY WT.		52352500	9802017	6	26423000	4437441	6	.053
LIVER % BODY WEIGHT		3.5	.4	4	3.2	.1	4	.445

TABLE 2.5.1. OLD MALE ACUTE BODY WEIGHT

VARIABLE	TIME HOUR	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
BODY WEIGHT								
ACUTE	0	526.3	-	1	422.7	115.2	2	-
	0.5	523.8	-	1	420.6	114.8	2	-
	1.0	518.6	-	1	418.0	113.1	2	-
	1.5	516.4	-	1	416.8	113.4	2	-
	2.0	514.0	-	1	414.9	113.6	2	-
	2.5	511.9	-	1	414.7	113.3	2	-
	3.0	512.1	-	1	414.6	113.3	2	-
	3.5	510.5	-	1	413.5	112.2	2	-
	4.0	510.9	-	1	411.8	111.5	2	-
	5.0	510.4	-	1	409.2	113.9	2	-
	6.0	510.0	-	1	396.9	106.6	2	-
	9.0	509.2	-	1	392.7	105.3	2	-
	12.0	506.5	-	1	389.8	104.7	2	-
	24.0	497.2	-	1	381.7	101.2	2	-
	48.0	522.8	-	1	397.5	102.5	2	-

TABLE 2.5.2. OLD MALE CHRONIC BODY WEIGHT

VARIABLE	TIME DAY	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT								
CHRONIC	3	516.8	-	1	402.8	101.0	2	-
	4	524.0	-	1	401.8	99.6	2	-
	5	523.2	-	1	408.5	108.5	2	-
	6	515.4	-	1	408.0	108.0	2	-
	7	515.1	-	1	408.9	111.5	2	-
	8	517.0	-	1	402.6	104.3	2	-
	9	523.4	-	1	406.7	106.7	2	-
	10	525.2	-	1	409.6	107.5	2	-
	11	521.3	-	1	412.3	105.8	2	-
	12	519.3	-	1	411.4	106.4	2	-
	13	524.1	-	1	411.7	103.0	2	-
	14	519.2	-	1	414.7	111.1	2	-
	15	525.5	-	1	395.7	97.9	2	-
	16	513.4	-	1	401.0	103.3	2	-
	17	516.1	-	1	106.8	100.6	2	-
	18	515.7	-	1	107.6	105.3	2	-
	19	522.0	-	1	107.4	99.3	2	-
	20	521.2	-	1	405.6	101.9	2	-
	21	526.4	-	1	411.6	109.5	2	-
	22	521.9	-	1	409.3	107.1	2	-
	23	523.7	-	1	401.1	106.2	2	-
	24	524.5	-	1	395.5	103.5	2	-

TABLE 2.5.3. OLD MALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
PCV	HOUR							p value t-test
ACUTE	0.0	48%	-	1	.465	.005	2	-
	0.5	46%	-	1	.460	.010	2	-
	1.5	49%	-	1	.420	.010	2	-
	3.0	49%	-	1	.380	.020	2	-
	12.0	45%	-	1	.445	.035	2	-
	24.0	47%	-	1	.425	.035	2	-
	48.0	45%	-	1	.395	.035	2	-
CHRONIC	DAY							
	6	49%	-	1	.425	.015	2	-
	13	50%	-	1	.475	.005	2	-
	20	52%	-	1	.465	.015	2	-
	24	50%	-	1	.465	.005	2	-

TABLE 2.5.4. OLD MALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
ESR	HOUR							p value
								t-test
ACUTE	0.0	.068	-	1	.064	.045	2	-
	0.5	.500	-	1	.163	.163	2	-
	1.5	.091	-	1	.051	.031	2	-
	3.0	.130	-	1	.134	.123	2	-
	12.0	.093	-	1	.141	.076	2	-
	24.0	.085	-	1	.111	.020	2	-
	48.0	.091	-	1	.101	.008	2	-
CHRONIC								
	DAY							
	6	.533	-	1	.375	.208	2	-
	13	.638	-	1	.336	.276	2	-
	20	.532	-	1	.307	.171	2	-
	24	.383	-	1	.423	.377	2	-

TABLE 2.5.5. OLD MALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance p value t-test
WBC MOBILITY	HOURL							
ACUTE								
	0.0	.000	-	1	.000	.000	2	-
	0.5	.250	-	1	.063	.063	2	-
	1.5	.250	-	1	.000	.000	2	-
	3.0	.000	-	1	.000	.000	2	-
	12.0	.000	-	1	.000	.000	2	-
	24.0	.000	-	1	.000	.000	2	-
	48.0	.380	-	1	.190	.190	2	-
CHRONIC								
	DAY							
	6	.500	-	1	.375	.375	2	-
	13	.250	-	1	.250	.000	2	-
	20	.750	-	1	.630	.250	2	-
	24	.000	-	1	.188	.063	2	-

TABLE 2.5.6. OLD MALE POST-MORTEM

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
% WATER	POST MORTEM							p value t-test
HEART		76.8	-	1	77.0	.690	2	-
LUNG		80.4	-	1	80.9	.695	2	-
LIVER		67.4	-	1	68.5	.345	2	-
PERFUSED LIVER		74.1	-	1	75.8	.570	2	-
LIVER HOMOGENATE		97.5	-	1	97.7	.265	2	-
HOMOGENATE DRY WT.		.005	-	1	.005	.001	2	-
LIVER CL/DRY WT.			-	1			2	-
LIVER % BODY WEIGHT		4.1	-	1	4.1		2	-

TABLE 2.6.1. OLD FEMALE ACUTE BODY WEIGHT

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
BODY WEIGHT	hour							p value t-test
ACUTE	0	290.1	5.6	5	280.0	10.0	4	.418
	0.5	288.5	5.4	5	278.1	9.2	4	.371
	1.0	286.6	5.4	5	277.0	9.3	4	.411
	1.5	285.1	5.2	5	275.1	9.6	4	.400
	2.0	284.2	5.2	5	274.9	9.2	4	.418
	2.5	283.5	5.1	5	274.9	9.1	4	.449
	3.0	282.6	5.0	5	274.6	9.3	4	.486
	3.5	282.1	4.8	5	273.8	9.4	4	.469
	4.0	281.5	5.0	5	273.0	9.1	4	.451
	5.0	280.1	4.8	5	270.2	8.0	4	.339
	6.0	279.5	5.1	5	268.0	8.1	4	.284
	9.0	276.6	5.2	5	262.5	8.3	4	.206
	12.0	274.3	5.5	5	259.9	8.6	4	.210
	24.0	267.2	5.7	5	254.4	7.6	4	.227
	48.0	285.3	6.6	5	260.9	6.3	4	.236

TABLE 2.6.2. OLD FEMALE CHRONIC BODY WEIGHT

VARIABLE	TIME DAY	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
BODY WEIGHT								
CHRONIC	3	283.9	5.3	5	267.4	13.1	4	.311
	4	282.4	3.7	5	269.1	14.4	4	.431
	5	285.9	4.3	5	268.6	14.3	4	.320
	6	287.4	4.8	5	270.4	14.0	4	.319
	7	286.3	5.1	5	268.0	13.8	4	.286
	8	287.1	5.8	5	270.1	14.4	4	.336
	9	288.8	6.1	5	270.7	15.4	4	.336
	10	289.5	6.2	5	267.9	16.2	4	.283
	11	288.9	5.9	5	271.3	14.9	4	.334
	12	291.3	6.7	5	274.1	16.8	4	.394
	13	292.7	7.0	5	274.8	15.1	4	.340
	14	292.5	6.7	5	274.5	14.8	4	.327
	15	289.4	6.9	5	278.0	15.1	4	.527
	16	292.1	6.5	5	278.4	12.5	4	.378
	17	293.1	6.2	5	280.8	11.2	4	.382
	18	289.5	7.3	5	284.6	10.5	4	.715
	19	291.9	6.8	5	286.4	9.9	4	.559
	20	294.3	7.3	5	286.1	11.7	3	.585
	21	289.6	7.3	5	283.6	11.5	3	.673
	22	294.0	8.1	5	282.7	13.1	3	.510
	23	288.1	5.3	5	284.6	12.1	3	.811
	24	292.5	8.5	5	284.2	13.9	3	.637

TABLE 2.6.3. OLD FEMALE ACUTE AND CHRONIC PCV

VARIABLE	TIME	SALINE	± S.E.M.	n	LPS	± S.E.M.	n	Significance
PCV	HOUR							p value t-test
ACUTE	0.0	45%	1.7	5	44	.9	4	.778
	0.5	45%	1.9	5	44	1.1	4	.654
	1.5	45%	1.8	5	39	3.1	4	.181
	3.0	44%	1.5	5	39	.6	4	.020
	12.0	43%	.9	5	43	.9	4	.501
	24.0	43%	1.3	5	44	1.5	4	.447
	48.0	42%	1.1	5	41	1.1	4	.652
CHRONIC	DAY							
	6	46%	1.9	5	42	1.5	4	.358
	13	44%	1.5	5	46	1.3	4	.962
	20	47%	2.4	5	46	.9	3	.728
	24	45%	1.3	5	47	.6	3	.264

TABLE 2.6.4. OLD FEMALE ACUTE AND CHRONIC ESR

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
ESR	HOURLY							p value
								t-test
ACUTE	0.0	.109	.038	5	.116	.045	4	.917
	0.5	.187	.038	5	.163	.090	4	.816
	1.5	.146	.045	5	.100	.023	4	.403
	3.0	.089	.020	5	.042	.015	4	.103
	12.0	.129	.052	5	.089	.017	4	.498
	24.0	.087	.030	5	.115	.027	4	.516
	48.0	.203	.045	5	.213	.064	4	.905
CHRONIC								
	DAY							
	6	.189	.037	5	.292	.093	4	.363
	13	.184	.043	5	.409	.141	4	.210
	20	1.87	.059	5	.278	.118	3	.540
	24	.175	.077	5	.301	.132	3	.464

TABLE 2.6.5. OLD FEMALE ACUTE AND CHRONIC WBC MOBILITY

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance
WBC MOBILITY	HOURL							p value t-test
ACUTE								
	0.0	.100	.061	5	.189	.082	4	.418
	0.5	.226	.100	5	.031	.031	4	.126
	1.5	.126	.056	5	.094	.060	4	.706
	3.0	.025	.025	5	.031	.031	4	.881
	12.0	.151	.074	5	.090	.090	4	.618
	24.0	.126	.126	5	.313	.157	4	.389
	48.0	.226	.140	5	.469	.241	4	.425
CHRONIC								
	DAY							
	6	.776	.350	5	.408	.156	4	.377
	13	.614	.157	5	.500	.177	4	.646
	20	.672	.165	5	1.793	.763	3	.277
	24	.326	.050	5	.753	.373	3	.371

TABLE 2.6.6. OLD FEMALE POST-MORTEM

VARIABLE	TIME	SALINE	+ S.E.M.	n	LPS	+ S.E.M.	n	Significance p value t-test
% WATER	POST MORTEM							
HEART		76.4	.271	5	65.2	.111	3	.862
LUNG		80.7	.166	5	80.7	.301	3	.994
LIVER		68.5	.289	5	68.4	.186	3	.942
PERFUSED LIVER		75.1	.198	5	75.1	.570	3	.986
LIVER HOMOGENATE		97.6	.107	5	97.6	.110	3	.911
HOMOGENATE DRY WT.		.005	.000	5	.005	.000	3	.756
LIVER CL/DRY WT.		41531900	22215300	5	45975100	51954800	3	.899
LIVER % BODY WEIGHT		3.2	.257	5	4.0	1.0	3	.356

CHAPTER 3

CONCLUSION

The increase in WBC and liver CL (due to free radicals) in the old male and female rats (Fig. 2.5.1 and 2.5.2, and 1.8.1 and 1.8.2, respectively) support the hypothesis that exposure to *E. coli* LPS in a open environment produces priming of the immune system cells to respond more dramatically to the immune probe PMA.

The hypothesis that the female rats' immune system is less affected by the LPS exposure when compared to old male rats, may be supported by the findings that: 1) the female rats' maintain their WBC mobility in old age (Fig. 1.4.1 and 1.4.2) and have a smaller increase in liver CL (Fig. 1.8.1 and 1.8.2).

The fact that there are no long-term differences in WBC CL, and WBC count in middle-aged and old rats, and that the long-term differences in WBC mobility, PP, and ESR are found in young males may be an indication that there are immune suppressive proteins in middle-aged and old rat plasma similar to that found by Delpierre et al. (1980) in the old cow extract (OCE).

The independent variable age has the strongest influence on the measured parameters, as is apparent from chapter 1. The untreated rats show increases in WBC CL (Fig. 1.1.1 and 1.1.2) and body weight (Fig. 1.7.1 and 1.7.2), and decreases

in time-to-peak chemiluminescence (Fig. 1.2.1 and 1.2.2), WBC counts (Fig. 1.3.1 and 1.3.2), WBC mobility (Fig. 1.4.1 and 1.4.2), plasma protein concentration (Fig. 1.5.1 and 1.5.2), and packed cell volume (Fig. 1.6.1 and 1.6.2). The increase in liver CL (Fig. 1.8.1 and 1.8.2) may also be related to the effect of increased exposure to LPS over the aging process and not due to probing of the immune system with the I.P. injection LPS 24 days earlier, since there was no difference between the LPS and saline treated liver CL on day 24.

The primary gender effect is on longevity. Only three males survived into old age. Within the surviving male rats there is an increase in the WBC count (Fig. 1.3.1), packed cell volume (Fig. 1.6.1), body weight (Fig. 1.7.1), and liver weight (Tables 2.1.6, 2.3.6 and 2.5.6) as a percent of body weight when compared to the old female rats.

The results in chapter 2 indicate that the acute effects of LPS treatment were both a decrease and then an increase in rectal temperature (Figures 2.2.1-2.4.2), WBC TTP (Figures 2.8.1-2.10.2), WBC count (Figures 2.11.1 to 2.13.2), plasma protein concentration (Figures 2.14.1 to 2.16.2), and packed cell volume (Figures 2.17.1 to 2.19.2). However, LPS treatment effected only an increase in WBC CL (Figures 2.5.1 to 2.7.2). LPS treatment had variable long-term effects on rectal temperature (Figures 2.20.1 to 2.22.2), increased PP (in young male rats only, Fig. 2.25),

decreased WBC mobility (Figures 2.23 and 2.24), increased ESR (Figures 2.26-2.27), and decreased packed cell volume (Figures 2.28-2.31). These changes may be due to age-related changes in the inflammatory response.

Unique findings

The WBC mobility findings (Figures 1.4.1-1.4.2 and 2.23-2.24) that show a decrease in the ability of male rats WBC to move (Fig. 1.4.1) has not been documented in the literature. The long-term effects (days 3-24) on rectal temperature (Figures 2.20.1 to 2.22.1), WBC mobility (Figures 2.23 to 2.24), plasma protein (Fig. 2.25), erythrocyte sedimentation rate (Figures 2.26 to 2.27), and packed cell volume (Figures 2.28-2.31) have not been reported on prior to this study. The post-mortem findings that the liver CL (Figures 1.8.1 and 1.8.2) and perfused dry weight (Tables 2.1.6, 2.3.6 and 2.5.6) increase with age, and that there is a gender difference such that old male rats have a higher liver CL than do the old female rats, have not been previously reported. It also appears that the immune response to LPS shows more similarity than diversity when comparing the male and female rats and the young, middle and old age groups. Therefore, it can be concluded that LPS treatment eliminates many of the age and gender differences seen in the untreated rats.

CHAPTER 4

DISCUSSION

The changes in the general physiological responses to *E. coli* LPS need further study. Inflammation is a complicated process that involves the interaction of white blood cells (monocytes, tissue macrophages, PMNs, T- and B-lymphocytes) (Le and Vilcek, 1987), soluble mediators (e.g., IL-1, IL-6, IL-8, TNF- α , and the kinins) (Miller and Sims, 1986; Epstein, 1987; Akira et al., 1990; Cerami, 1992; Burke-Gaffney and Keenan, 1993; Cheronis and Whalley, 1993), and the vascular components such as the endothelial cells, Intracellular Adhesion Molecule-1 (ICAM-1), and Extracellular Adhesion Molecule-1 (ECAM-1) (Akira et al., 1990; Hawryloxicz et al., 1991; Cheronis and Whalley, 1993).

The body core temperature (T°_b , measured by rectal probe) changes in opposite directions in the control and LPS-treated groups (Figures 2.2.1 through 2.4.2). Also, within each treatment group the T°_b changes in opposite directions, between 0-6 and 6-48 hours, to various degrees in each gender and age group. There is an initial hypothermia that may be related to changes in prostaglandin D2 (Tanamoto, 1990) or TNF- α (Heggars and Robson, 1985; Morrow et al., 1993) or both. The hypothermia corresponds to the time course of TNF- α release following LPS treatment (Michie et al., 1988). The hyperthermia may be related to

IL-1 (Le and Vilcek, 1987) or IL-6 (Morrow et al., 1993) and hypothalamic PGE₂ secretion (Dinarello and Bernheim, 1981; Heggars and Robson, 1985). The release of IL-1 has been shown to be triggered by the autocrine feedback of TNF- α on the monocyte about 1.5-2.5 hours after monocyte and macrophage stimulation by LPS (Michie et al., 1988). The timing of IL-1 release corresponds to the fever response seen at this time in Figures 2.2.1 to 2.4.2. The fact that the older rats have a prolonged hypothermia (Figures 2.3.1 and 2.3.2) and do not achieve fever may be due to: 1) a prolonged secretion of TNF or PGD₂, 2) decreased PGE₂ production, 3) decreased release of IL-1 from the WBCs or 4) decreased sensitivity of the hypothalamus to the circulating IL-1, or other factors.

Knook and Brouwer (1989), injected various doses of endotoxin, 10-40 mg/kg, into female rats of all ages. The effect on body temperature was similar to that found in this study using 0.5 mg/kg, i.e., the older rats suffered a prolonged hypothermia and failed to develop a fever (Knook and Brouwer, 1989). Johansen et al. (1983), report that the optimum body temperature for maximal free radical production is 40 °C, and that free radical production decreases at temperatures greater or less than 40 °C. In spite of the lack of fever in the old rats, when Figures 2.5.1 through 2.7.2 are compared, the old rats show an increase in WBC free radical production as measured by chemiluminescence.

Since the old rats CL is already elevated, the lower body temperature or lack of fever may be advantageous rather than detrimental, since CL would be even higher if the enzymes were provided an optimum operating temperature of 38-40 °C and more free radical damage would occur.

Recent reports have begun to detail how LPS alters the activation state of the phagocytic cell lines (Allen et al., 1972; Johnston, 1985; Hakkert et al., 1991). The monocyte "orchestrates" the free radical anti-bacterial response to LPS (Altman, 1978; Klempner, 1979; Maizel and Lachman, 1984; Raetz et al., 1991; Morrow et al., 1993). LPS is bound by a circulating binding protein (Schumann, 1990) and this complex then binds to the differentiation antigen CD14 (Schumann, 1990) on the surface of the monocyte which, in turn, activates the macrophage (Wright and Jong, 1986; Schumann, 1990).

The activated macrophage internalizes the complex, processes it, and re-presents the antigen on its plasma membrane in conjunction with the Type II major histocompatibility complex (MHC-II) (Ohmann et al., 1988). As the antigen is being processed and re-presented the macrophage begins to release tumor necrosis factor-alpha [TNF- α , a.k.a. cachectin (found to be the same molecule by amino acid and cDNA sequencing) (Beutler and Cerami, 1987)] (Schumann, 1990; Skau, 1992). TNF- α molecules associate to form non-covalent multimers which then bind to the membrane

receptors (Warren, 1993). The autocrine binding of TNF- α to a monocyte or macrophage induces further release of TNF- α and initiates the release of the endogenous pyrogen interleukin-1 (IL-1) (Dinarello and Mier, 1987; Beutler and Cerami, 1988; Warren, 1993), interferon gamma (IF- γ), complement fragments C4 and C5, prostaglandins, and cytokines (Kuehl and Egan, 1984; Dinarello and Mier, 1987). TNF- α is, further, responsible for local PMN activation, endothelial cell activation, leukocyte recruitment into the inflammatory site, release of WBCs from the bone marrow, release of erythropoietin from the kidney and catabolism of bone, cartilage and matrix (Dahinden et al., 1983; Warren, 1993). Sasada et al. (1983) showed that macrophages activated in vitro by LPS and then stimulated with PMA produced more free radicals than do normal resident macrophages (Klebanoff, 1970; Klebanoff, 1982; Korchak, 1984; Kitahora, 1988). The neutrophils are stimulated to diapedese and migrate, following the C4 and C5 concentration gradient (Tranquillo et al, 1988), to the site of inflammation (Ulich, 1989). The activation of PMNs was demonstrated by Johnston et al. (1983). Activated PMNs show increased secretory activity as well as increased free radical production (Jandl et al., 1978; Thelen et al., 1993). Figures 2.5.1 to 2.7.2 probably demonstrate the very

acute (1-4 hour) effects of LPS on the CL (free radical production of circulating monocytes and PMNs).

TNF- α and IL-1 may also effect CL in an indirect fashion. TNF- α significantly reduces the incorporation of plasma iron into newly synthesized RBCs producing an iron deficiency anemia (Klempner et al., 1977; Moldauer et al., 1989). Loria, Hershko and Konjin (1979) found that there is an increase in the circulating serum ferritin in elderly males and female subjects with iron deficiency anemia; elderly females had ferritin concentrations lower than the elderly males. Iron can be released from ferritin by superoxide and the other organic radicals (Aust, 1987). In turn, non-heme iron is responsible for the reduction of molecular oxygen to superoxide (Massey et al., 1969; Borg and Schaich, 1987). Thus, an increase in circulating plasma iron concentrations may account for, or contribute to, the increased CL in the older rats and for the fact that older males have a higher CL than do the older female rats (Figure 2.6.1 and 2.6.2) (Minnotte et al., 1987).

LPS does not have long-term effects on WBC CL, in spite of the redistribution of LPS (Freudenberg et al., 1982). A possible mechanism for this is that the macrophage and its TNF secretion are tightly regulated at many levels of action. The macrophage is deactivated by IL-10 and macrophage deactivating factor (Bogdan et al., 1991), TNF- α secretion is controlled by negative feedback of IL-6 (Akira

et al, 1990), TNF- α gene expression is regulated by both transcription and post-transcriptional mechanisms (Ulich et al., 1989), and TNF- α receptors are down regulated by IL-1 (Burke-Gaffney and Keenan, 1993), all within the first 6 hours post LPS.

The transient leukopenia and subsequent leukocytosis as seen in Fig. 2.10.1 through 2.12.2 parallel the suggested time course of TNF- α (Michie et al., 1988) and IL-1 release (Michie et al., 1988; Clark et al., 1991), of 0.5-3 and 3-24 hours, respectively. TNF- α has been shown to induce a transient neutropenia, peaking 2.5 hours post injection followed by a neutrophilia paralleling the fever which is induced by IL-1 (Le and Vilcek, 1987; Moldawer et al., 1989; Ulich et al., 1989). The neutropenia may also be due to the granulocyte-colony-stimulating factor (Boxer, 1992). These cytokines may therefore be responsible for the leukopenia seen in Figure 2.10.1 to 2.12.2

The proposed mechanism for the TNF- α induced leukopenia is an increase in the WBC margination and PMN migration to the site of inflammation (Ulich et al., 1989). Although TNF- α promotes endothelial cell adhesion properties, this is primarily an IL-1 effect (Ulich et al., 1989; Hakkert, et al., 1991; Hawryloxicz et al, 1991). TNF- α induces WBC release from the bone marrow, but at a much slower rate than

the effect of TNF- α on WBC margination (Ulich et al., 1989). The leukocytosis is likely due to both TNF- α and IL-1 (Smith et al., 1987; Kampschmidt, 1980). The primary TNF- α effect on leukocytosis is a time dependent diminution of the margination effect of TNF- α . In other words, the margined WBCs are now demargined and re-enter the circulating pool of cells (Ulich et al., 1989).

The structure of the microcirculation filtration system varies from tissue to tissue depending on the local requirements (Shepro, 1980). For example the brain, skin, muscles and lungs must have a vasculature that will restrict large increases in fluid volume (Ryan and Majno, 1977; Shepro, 1980). Yet, other tissues have large fenestrae that accommodate a large flux of fluid and the transmigration of white blood cells, e.g., the open fenestrae of the kidneys, bone marrow, and lymph nodes, or fenestrae that are closed by a thin diaphragm as found in the endocrine and exocrine glands, (Ryan and Majno, 1977; Shepro, 1980).

An increase in the normal vascular leakage (transudation) secondary to an inflammatory process is termed exudation and results in the swelling called *tumor*. Trans-endothelial movement of fluids (transudation) occurs principally at the level of the capillary and small venule (Miller and Sims, 1986). The maintenance of healthy extravascular tissue is dependent upon the transudation of

plasma and proteins from the bloodstream into the interstitial tissue (Ryan and Majno, 1977; Ganong, 1985). This normal fluid movement is controlled by a balance of hydrostatic and oncotic forces (Shepro, 1980). The force driving fluid out of the vasculature, estimated to be about 33 mm Hg, is due to the coupling of intravascular hydrostatic pressure (averaging about 23 mm Hg) and the oncotic force of the interstitial tissue (about 10 mm Hg) (Ryan and Majno, 1977). There is an opposing 26-29 mm Hg force comprised of the oncotic pressure due to the plasma proteins (about 25 mm Hg) and the hydrostatic pressure of the interstitial tissue (about 1-4 mm Hg) which forces fluids back into the blood stream (Ryan and Majno, 1977). An additional variable is the permeability of the vessel wall and is represented by a constant K_1 (Shepro, 1980). Since the force driving fluid out overwhelms the force moving the fluids into the bloodstream there is a small, but critically important, transudation or net flow of an ultrafiltrate out of the bloodstream (Ryan and Majno, 1977; Ganong, 1985).

The protein content of a *transudate* is normally relatively low (0.2 to 0.5 gm/100 ml) (Ryan and Majno, 1977). However, the protein content of an *exudate*, the fluid shift during inflammation, is high (2-5% or higher) (Ryan and Majno, 1977). The reason for the change in composition of the fluid leaving the capillary is that there

is a heterogeneous change in the integrity of the vessel wall permeability (K_1) of the venules and at the venular end of capillaries (Miller and Sims, 1986). The heterogeneous permeability is apparently correlated with sites of effectors and receptors for Bradykinin (Miller and Sims, 1986). There are at least two mechanisms involved in changing the permeability of the capillary. The first is the damage caused by the inflammatory agent itself, i.e., direct injury by foreign bodies (Deuschle and Weser, 1985). The other mechanism is due to the inflammatory mediators such as IL-1 α/β , TNF- α and IF- γ (Burke-Gaffney and Keenan, 1993) released by the tissues of the body in response to the damage (Clark, 1991). A post cytokine-release protein-rich exudate may account for the decrease in circulating plasma protein concentrations seen acutely, in Figures 2.13.1 to 2.15.2, since the increased vascular permeability would allow proteins to leak into the interstitial space. Although drinking was not measured, there may be an increase in the water intake of the LPS-treated rats which would also decrease the plasma protein concentration.

Bradykinin and kallidin (Lys-Bradykinin), collectively known as the kinins, are mediators of the cell-independent inflammatory response to LPS. The kinins are believed, by some researchers, to be pivotal regardless of the cause of the inflammation (Cheronis and Whalley, 1993). The effects of kinins on the vascular endothelium and smooth muscle, due

to their binding to receptors termed BK₁ (upregulated during an inflammatory response, perhaps by TNF and or IL-1) and BK₂ (present on all normal tissue), produce endothelial retraction and vasodilatation as a part of the inflammatory response and results in *rubor, tumor, and calor*; the result of kinin action on the β 2 receptors of C-terminal nerve fibers, which release substance-P, effects *dolor* (Cheronis and Whalley, 1993).

Although kinins are the mediators of cell independent inflammation they also stimulate circulating monocytes and PMNs to release various cytokines which have further effects on the endothelial cells and the integrity of the extracellular matrix (Cheronis and Whalley, 1993). Bradykinin and kallidin are activated by two separate enzymatic pathways. The first takes place in the circulation, and the second involves membrane bound proteins and activation enzymes (the second pathway is, as yet, poorly understood) (Cheronis and Whalley, 1993).

As a response to the presence of LPS in the circulation, Factor XII is activated which then cleaves plasma prekallikrein into kallikrein which, in turn, clips a high molecular weight (HMW) kininogen to release an active, preformed, Bradykinin which is a BK₂ agonist (Cheronis and Whalley, 1993). The BK₂ agonist of the membrane bound pathway is released when tissue kallikrein stimulates a low molecular weight (LMW) kininogen to cleave kallidin. Then

either kininase I (carboxypeptidase-N of -M) or II (neutral peptidase of angiotensin converting factor) cleave the BK₂ agonists into their inactive metabolites, of which the des-arg derivatives are BK₁ agonists (Cheronis and Whalley, 1993).

An increased ESR has been positively correlated with an IL-6-induced (Akira et al., 1990) increase in the acute phase reactants such as C-reactive protein, fibrinogen (Kenney et al., 1985; Berlinger et al., 1988; van der Poll et al., 1990), α -1 anti-chymotrypsin (Chard, et al., 1988), and negatively correlated with increased albumin concentration. Apparently higher molecular weight C-reactive protein, fibrinogen and alpha-1 anti-chymotrypsin (Chard et al., 1988) increase the RBC cell aggregation, while the smaller spherical shape of albumin decreases aggregation (Berlinger et al., 1988; Reinhart, 1988). Leukocyte adhesiveness/aggregation (LAA) is a manifestation of the acute phase reaction perhaps due to the effects of TNF- α (Berlinger et al., 1988). In patients with rheumatoid arthritis, IL-1 β concentrations correlated positively with increased ESR (Eastgate et al., 1988). Therefore, the elevation in ESR seen between days 6 and 24 in young male (Figure 2.26) and old female rats (Figure 2.27) may be due to small amounts of IL-1 being released secondary to the re-release of LPS from the liver (Freudenberg et al., 1982).

LONG-TERM EFFECTS OF LPS

In the present study, male rats' PCV was greater than the females and older rats had lower PVC's than younger rats (Figures 2.17.1-2.19.2 and 2.28-2.31). When present chronically, in vivo LPS and/or TNF- α induce a decrease in tail blood PCV (as seen in Figures 2.27 to 2.28). According to Moldawer et al. (1989) RBC count and hemoglobin content decrease, but there is no change in mean corpuscular volume or mean corpuscular hemoglobin content.

The probable TNF- α -stimulated leak of protein disappears by day 6 and is followed by an increase in circulating plasma protein concentration (Figures 3.24-3.26). The review of the microcirculation by Shepro (1980) points out that in 1926 A. Krogh documented that only a fraction of the microvascular system is perfused at any one time, and that blood flow is intermittent in the resting state, and increases anywhere from 5 to 100 times in exercising muscle, depending on the level of exertion (Shepro, 1980). During the acute phase of the protocol, the LPS treated rats became lethargic at 1-1.5 hours and remained less active than the control rats through day 1. During this inactive period the peripheral vasculature does not need to be actively perfused. On day 2 when activity levels returned to normal, there was a concomitant increase in muscle blood flow. Vessels that were not perfused when the inflammatory mediators were in high concentration, and

were therefore not leaky, would then be opened. The redistribution of the blood flow to a large, non-leaky, vascular bed may have contributed to the retention of plasma protein resulting in the increased plasma protein concentrations during the chronic phase of the protocol. Miller and Sims (1986) report that there is a decrease in vascular leakage secondary to cooling, therefore with the return of the body temperature to normal, there will also be a decrease in protein exudation.

Two non-vascular explanations for the increase in plasma protein are: 1) since the liver is still processing and re-releasing LPS (Freudenberg et al., 1982), the liver may still be producing PP at a higher rate, and 2) since the Amago refractometer technique was non-specific for the type of protein being measured the increase could be due to methylhistamine (Uauy et al., 1978) and, therefore, related to the cachectic, muscle wasting effect of TNF- α (Uauy et al., 1978; Le and the Vilcek, 1987). Future studies need to be done to differentiate between the subsets of circulating proteins.

Knook and Brouwer (1989) injected various doses of endotoxin, 10-40 mg/kg, into female rats of all ages and found a decrease in endotoxin clearance from the circulation apparently due to an age-related decline in the old females' Kupffer cells ability to clear LPS. The old females' elimination of radioactively-labeled LPS from the

circulation was prolonged, but there was no significant decrease in the number of Kupffer cells isolated per gram of tissue, from the liver. In contradiction, Wake et al. (1989) report that post-LPS, the liver shows an increase in the number of Kupffer cells, and that the increase is due to both local proliferation and migration of monocytes from the bone marrow. This seemingly conflicting information may be explained by the dose of LPS given to the rats by Knook and Brouwer. Knook and Brouwer were studying systemic shock, and very high doses (10-40 mg/kg) of endotoxin produced a high mortality rate due to induced systemic coagulation which apparently was lethal to many older rats. And in the survivors, since the protocol did not extend beyond 24 hours, perhaps there was not enough time for increased WBC immigration into the liver to be noticed. In this study the lower dose of 0.5 mg/kg LPS and the longer survival time (24 days) may have allowed for enough time to induce monocyte immigration to increase the liver phagocyte population and hence to increase the perfused liver dry weight.

The increase in liver CL in the old rats in this experiment (Figures 1.1 to 1.2 and 2.4.1. to 2.6.2) may be explained by the repeat exposure hypothesis, since rats raised in a pathogen-free aging colony showed a decrease in WBC CL with increasing age (Davila et al., 1990). Animals raised in the Biomedical Laboratory Animals Facility were raised in an open environment where the rats would

ultimately come in contact with *E. coli* in the air, food and water. Furthermore, repeated transmural release LPS from the gut and uptake by the liver (Freudenberg et al., 1982) may maintain an increased number, and perhaps a continuous state of activation, of the Kupffer cells in the livers of old rats. Since Kupffer cells adhere to the hepatic sinusoids and the space of Disse (perivascular space) by extending cytoplasmic processes that extend underneath or embed in the basement membrane (Wake et al, 1989), they are not subject to depletion by perfusion. An increase in Kupffer cell or macrophage numbers could account for the decreased water content (increased dry weight) of the perfused liver of old rats.

Another possible explanation for the increase in liver CL is the finding that the liver of old male (24-26 months) Wistar rats shows a decrease in total superoxide dismutase (SOD) and magnesium SOD (mn-SOD), but no change in copper-zinc SOD (Cu/Zn-SOD) (Danh et al., 1983). Mn-SOD is the dismutase that is found in the mitochondrial matrix of plants and animals (Danh et al., 1983). One focus of the free radical theory of aging is on the mitochondria as the "biological clock" (von Zglinicki, 1987). This hypothesis incorporates part of the membrane theory of aging which involves the decrease in water content that is apparent in cells in rat heart, liver and brain (von Zglinicki, 1987). The decrease in heart and liver water content has been

shown to be due to water loss in the mitochondria due to an increased permeability of the inner mitochondrial membrane. The author equated the old liver mitochondrial water content to that of bone (von Zglinicki, 1987). The decrease in water content decreases the osmotic reflection coefficients for organic ions, limiting diffusion of intramitochondrial metabolites, but increases the electrostatic interaction of polarized molecules (von Zglinicki, 1987). Water loss increases the ionic strength above 600 mmol/kg water, over 200 mmol/kg above the value that has been shown to increase steric hindrance and interferes with enzymatic function (von Zglinicki, 1987). Such changes will undoubtedly also affect the mitochondrial Mn-SOD function, but may also extend into the cytosol. The increase in mitochondrial membrane permeability would allow for an influx of K^+ (down its ionic concentration gradient). To maintain its membrane potential, the mitochondria would have to increase K^+ and other cation (e.g., Ca^{++} and Mg^{++}) efflux. An increase in cytosolic Ca^{++} would stimulate the xanthine and NADPH oxidase free radical production.

APPENDIX A

Animal comfort

The research animals (rats) for this study were bred and raised by the investigator, so as to insure their comfort and for practical reasons. Since stress causes the release of glucocorticoids, which suppress the immune response (Reinehart et al., 1974; Baker et al., 1976; Munster, 1985; Schaffner and Schaffner, 1987; Hogan and Vogel, 1988), it was imperative that the handling stress during the protocol be minimal. Improper handling unnecessarily increases the distress of the rats (signs of a frightened rat were eyes bulging outward, gnashing teeth, biting, and scratching). The following system was devised to decrease the rats' stress while allowing for observation of each rats' normal behavior before the rat became a part of this research project.

Once weaned, young rats were accustomed to being handled by the investigator on a daily basis. Each rats' normal waking and sleeping behavior was observed and evaluated so that any behavior which was out of the ordinary for that particular rat could be identified. For example, during the mid-morning and afternoon the rats were usually asleep, and the most common posture for rats housed in individual cages, with the overhead lights on, was with the head tucked under the abdomen. A less common, but frequent behavior was to

burrow under the bedding. To observe the rats' normal waking behavior and activity level, the rats' cage was opened at 5:00 A.M., seven days a week, for approximately 30 minutes. Each animal then had the freedom to exercise, ad lib, while exploring its surroundings (Duester, 1990).

Some typical behaviors were: 1) inactivity, sitting 2) climbing the wall of the cage and walking around the cage top, 3) climbing and exploring areas adjacent to the cage, 4) a combination of #2 and 3 and climbing up the underside of the cage top and perform back-flips onto the cage floor. During this exercise period the rats received a daily treat of several kernels of plain air-popped popcorn. This treat was always welcomed. Most rats stopped to eat the popcorn and resumed their activity later. Although some rats continued to explore, once they were back in a closed cage they promptly ate the popcorn. These activity sessions set certain routines for the rats that they seemed to enjoy as a diversion from cage life. Each day when the facility door was opened all the rats would run to the front of their cage, stand on their hind legs and poke their noses through the cage or bump the cage top. Rats that are frightened do not exhibit this behavior, frightened rats run to the back of the cage and cower there.

Daily for at least five days prior to the onset of the research protocol the rats were held in the investigator's lap and allowed to explore ad lib; and, also, become

accustomed to the lab equipment, insertion of the rectal probe, and being place in the closed weighing pan. Each rat was always lifted under the shoulders or around the waist (never by its tail) and carried to and from the work area on a clean towel. There was never any need to use any type of restraining device, so the rat was free to explore the work area, even during data collection.

The Wistar rat was chosen because of its gentle and cooperative nature. The male and female rats were large enough to allow for multiple blood samples without compromising its well being, yet small enough to have the rat unrestrained in the investigator's lap. Also, the function of the WBCs appear to be very similar in rats and humans.

Because of the inability to eliminate pain from the protocol every conceivable step was taken to minimize the pain by maximizing the care and comfort of the rat prior to and during the procedure. The rats were not anaesthetized for several reasons. First, the administration (injection) of anesthesia in and of itself can be a very disturbing event and once the anesthesia begins to reach the brain many rats become visibly frightened, i.e., the rats try to run away, cower in their cage, their eyes bulge, and often the rats violently shake their heads and gnash their teeth. These behaviors occur following the injection of anesthetic often enough to make me believe that this is not a

particularly kind thing to do to the rat on a repetitive basis. The effects of Halothane gas administration, observed at Tripler hospital, were symptoms of rat distress. Application of topical anesthesia (lidocane) to the tail, prior to and after cutting the tail produced extreme agitation in the rat which behaved as if it was in a great deal of pain, i.e., it ran around in circles chasing its tail, attempting to chew at the tail and squeaking continuously until the lidocane was washed off. Finally, this is a longitudinal study and would require daily anesthesia which is not only impractical, but would alter the entire physiologic response of the rat, but especially the WBCs, to LPS.

The literature reviewed report that the dose of E. coli LPS commonly given to rats ranges from 1 mg/kg (Smith, 1987) to 50 mg/kg (Chang et al., 1988). From behavioral observations, made at the indicated times pre- and post-injection for the control (saline) and test (LPS) rats, that neither the dosage of 0.5 mg/kg E. coli LPS (O55:B5, Sigma) nor the tail cut procedure, produce extreme discomfort nor altered the rats amiable behavior.

Pre-injection the tail tip was cut and blood was collected. The clipping of the tail may or may not even be noticed by the rat. Those rats that did react, reacted with a startle response very similar to that of a human who has just had their finger lanced for blood collection, i.e., a

jump and perhaps a squeak. When this response occurred the rat was comforted by rubbing and/or stroking the back of its head and back. Once the rat resumed its exploring behavior, usually within ten to fifteen seconds, the blood collection proceeded and the rat remained undisturbed, many rats actually watched the blood collection in a curious manner. During the blood collection the rats, more commonly, explored their surroundings, i.e., the experimenters lap or the adjacent lab bench. During the cutting of and subsequent blood collection from the tail no rat ever bit or scratched the investigator (in other labs the handling of their rats produced fear, psychological and/or physical pain and elicited biting and scratching behavior).

Once cut, the rats' tail was kept clean to impede infection (which never occurred in any of the tail-cuts in this experiment). The tail cut protocol is, in practical fact, less traumatic to the rats and, therefore less immunosuppressive, than the administration of general anesthesia or the surgical implantation of an arterial catheter (Munster, 1985).

After the rat was given either a LPS or saline injection I.P., it was placed back in its cage. The first one (1) to one and one-half ($1\frac{1}{2}$) hours after the injection both the saline and LPS rats were usually awake sitting and/or moving around the cage, occasionally they were curled up sleeping, behaving in a fashion typical of their pre-injection

behavior. At 1½ hours the LPS rat began to become lethargic, lying down in an uncharacteristic stretched-out posture, and about 50% have an episode of soft stool or diarrhea. During the temperature/body weight measurements the test (LPS) rat was extremely cooperative (often climbing onto the towel to be lifted out of the cage and climbing off of the towel into the weighing pan), far more so than the saline treated rat who appeared rather irritable. The irritability was perhaps due to the constant handling during a time when they would normally be asleep, because the irritability decreased as the intervening time increased.

On day 24, 30-65 mg/kg Sodium Pentobarbital was injected I.P. Each rat was held and comforted by stroking their head and back until the anesthesia took effect. This was to ensure that the animal was not distressed by the numbing effects of the anesthetic on the brain. It was observed that when the rat was placed back in the cage it attempted to move around, and as it became ataxic it often became distressed. Even when unconscious the distressed rats' eyes remained open, often bulging, and the tongue was protruding and clenched in the teeth. But the appearance of the rats that were held and comforted as the anesthesia took effect was different, these rats eyes were closed and the tongue was relaxed inside the mouth.

APPENDIX B

Heart, lung, and liver tissue was fixed in Bouin's Solution, the formula is as follows:

Saturated Picric Acid	75 ml
37% Formaldehyde	25 ml
Glacial Acetic Acid	5 ml

APPENDIX C

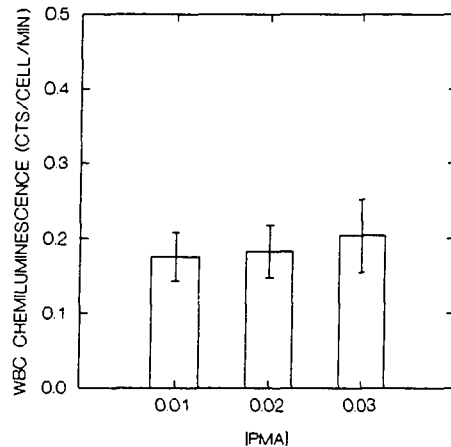
Sedimentation rate

PVC	Anticipated Sedimentation Rate (Wintrobe Tube)	Anticipated Sedimentation Rate (75 mm Capillary Tube)
9	82	56
10	79	54
11	76	52
12	73	50
13	70	48
14	67	46
15	64	44
16	61	42
17	58	40
18	55	38
19	52	36
20	49	34
21	46	32
22	43	30
23	40	28
24	38	26
25	36	25
26	35	24
27	32	22
28	30	21
29	28	20
30	26	18
31	24	17
32	22	16
33	20	14
34	18	13
35	16	11
36	14	10
37	13	9
38	12	8
39	11	8
40	10	7
41	9	6
22	8	6
43	7	5
44	6	4
45	5	3
46	4	3
47	3	2
48	2	1
49	1	1
50	0	0

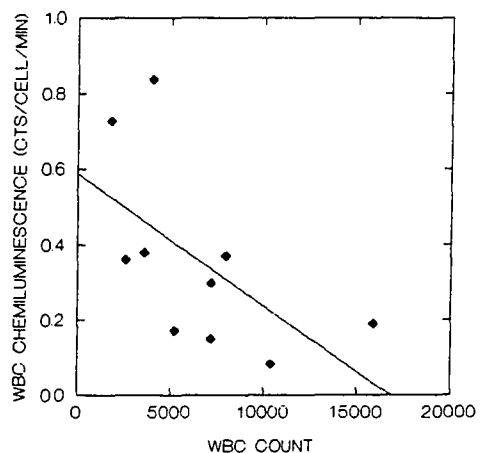
APPENDIX D

Control studies

To determine which effects were due to the treatment, i.e., I.P. injection of *E. coli* LPS, and insure that cell and/or stimulant concentrations were not influencing the results, the following experiments were conducted on untreated animals. First, cell concentrations were held constant and PMA concentrations were varied from 0.1 mg/ml to 0.3 mg/ml (one to three times normal stimulant concentrations).



The effect of varying the PMA concentrations ([PMA]) while holding the WBC concentrations constant.



The effect of increasing WBC concentrations on the counts per cell following 0.1 mg/cc PMA stimulation.

Changing the PMA concentration had no effect on the WBC CL. Varying the cell concentrations at a constant PMA concentration resulted in a decrease in CL as cell concentrations increased.

BIBLIOGRAPHY

- Akira, S., Hirano, T., Taga, T., Kishimoto, T. Biology of Multifunctional Cytokines: IL-6 and Related Molecules (IL-1 and TNF). The FASEB Journal. 4:2860-2867, 1990.
- Akiyama, Y., Griffith, R., Miller, P., Stevenson, G.W., Lund, S., Kanapa, D.J., Stevenson, H.C. Effects of Adherence, Activation and Distinct Serum Proteins on the In Vitro Human Monocyte Maturation Process. J. Leukocyte Biology, 43:224-231, 1988.
- Allen, R.C., Stjernholm, R.L., Steele, R.H. Evidence for the Generation of an Electronic Excitation State(s) in Human Polymorphonuclear Leukocytes and its Participation in Bactericidal Activity. Biochemical and Biophysical Research Communications. 47(4):679-684, 1972.
- Altman, J. Ins and Outs of Cell Signaling. Nature. 331:119-120, 1988.
- Aust, S.D. Sources of Iron for Lipid Peroxidation in Biological Systems. Oxygen Radicals and Tissue Injury. The UpJohn Company, pp. 27-32, 1988.
- Babior, B.M. Oxygen-Dependent Microbial Killing by Phagocytes. The N.E. J. of Med. 298(12):659-668, 1978.
- Baggiolini, M., Dewald, B. The Neutrophil. International Archives of Allergy and Applied Immunology. 76(suppl. 1):13-20, 1985.
- The Laboratory Rat Volume I, Biology and Diseases. Baker, H.J., Lindsey, J.R., Weisbroth, S.H., editors. Academic Press, San Diego, Ca., 1976.
- Beutler, B. and Cerami, A. The Common Mediator of Shock, Cachexia, and Tumor Necrosis Factor. Advances in Immunology. 42:213-231, 1988.
- Berlinger, S., Freid, M., Caspi, D. Weinberger, A., Yaron, M., Pinkhas, J. and Aronson, M. Evaluation of disease activity in rheumatic patients by leukocyte adhesiveness/aggregation. Annals of Rheum. Disease [62W] 47(6):458-462, June 1988.
- Bogdan, C., Vodovotz, Y., Nathan, C. Macrophage Deactivation by Interleukin 10. J. of Exp. Med. 174:1549-1555, 1991.

- Borg, D.C. and Schaich, K.M. Iron and Iron-Derived Radicals. Oxygen Radicals and Tissue Injury. The UpJohn Company, pp. 20-26, 1988.
- Boxer, L.A., Hutchinson, R., Emerson, S. Recombinant Human Granulocyte-Colony-Stimulating Factor in the Treatment of Patients With Neutropenia. Clin. Immunology and Immunopathology. 62(1):S39-S46, 1992.
- Brandt, D.B., Cathcart, E.S., Cohen, A.S. Studies of Immune Deposits in Synovial Membranes and Corresponding Synovial Fluids. J. Lab. & Clin. Med. 72(4): 631-647, October, 1968.
- Braude, A.I. Absorption, Distribution, and Elimination of Endotoxins and Their Derivatives. Bacterial Endotoxins, Landy, M., and Braun, W., editors. Rutgers University Press, 1964.
- Briheim, G., Stendahl, O., and Dahlgren, C. Intra- and Extracellular Events in Luminol-Dependent Chemiluminescence of Polymorphonuclear Leukocytes. Infection and Immunity. 45(1):1-5, July, 1984.
- Burke-Gaffney, A., and Keenan A.K. Modulation of Human Endothelial Cell Permeability by Combinations of Cytokines Interleukin-1 α/β , Tumor Necrosis Factor- α and Interferon- γ . Immunopharmacology. 25:1-9, 1993.
- Buys, S.S., Gren, L.H., Kaplan, J. Phorbol Esters and Calcium Ionophores Inhibit Internalization and Accelerate Recycling of Receptors in Macrophages. The J. of Biol. Chem. 262(27):12970-12976, September 25, 1987.
- Cadenas, E. Biochemistry of Oxygen Toxicity. Annual Reviews of Biochemistry. Annual Reviews, Inc., 1989, pp. 79-110.
- Campbell, E.J., and Campbell, M.A. Pericellular Proteolysis by Neutrophils in the Presence of Proteinase Inhibitors: Effects of Substrate Opsonization. The J. of Cell Biology. 106:667-676, March, 1988.
- Cartee, G.D. Aging Skeletal Muscle: Response to Exercise. Exercise and Sports Sciences Reviews, Vol. 22, 1994.
- Cerami, A. Inflammatory Cytokines. Clinical Immunology and Immunopathology. 62(1):S3-S10, 1992.

- Chang, S., Lauterburg, B.H., Voelkel, N.F. Endotoxin Causes Neutrophil-independent Oxidative Stress in Rats. *J. Appl. Physiol.* 65(1):358-367, 1988.
- Chard, M.D., Calvin, J., Price, C.P., Cawston, T.E., Hazelman, B.L. Serum Alpha-1 Anti-Chymotrypsin Concentration As a Marker of Disease Activity in Rheumatoid Arthritis. *Annals of Rheum. Disease* 47(8):665-671, August 1988.
- Cheronis, J.C., and Whalley, E.T. Kinin Receptor Antagonists. *Drug News and Perspectives.* 6(6):381-389, 1993.
- Cheson, B.D., Christensen, R.L., Sperling, R., Kohler, B.E., Babior, B.M. The Origin of the Chemiluminescence of Phagocytosing Granulocytes. *The J. of Clin. Invest.* 58:789-796, 1976.
- Clark, B.D., Bedrosian, I., Schindler, R., Cominelli, F., Cannon J.G., Shaw, A.R., and Dinarello, C.A. Detection of Interleukin 1α and 1β in Rabbit Tissues During Endotoxemia Using Sensitive Radioimmunoassays. *J. Appl. Physiol.* 71(6):2412-2418, 1991.
- Cromartie, W.J., et al. Arthritis in Rats After Systemic Injection of Streptococcal Cells or Cell Walls. *J. of Exp. Med.* 146:1585-1602, 1977.
- Dahinden, C., Galanos, C., Fehr, J. Granulocyte Activation by Endotoxin. I. Correlation Between Adherence and Other Granulocyte Functions, and Role of Endotoxin Structure on Biologic Activity. *The J. of Immunology.* 130(2):856-868, 1983.
- Danh, H.C., Benedetti, M.S., Dosert, P. Differential Changes in Superoxide Dismutase Activity in Brain and Liver of Old Rats and Mice. *J. of Neurochem.* 40:1003-1007, 1983.
- Davila, D.R., Edwards, III, C.K., Arkins, S., Simon, J., Kelley K.W. Interferon- γ -induced Priming From Secretion of Superoxide Anion and Tumor Necrosis Factor- α Declines in Macrophages from Aged Rats. *The FASEB Journal,* 4:2906-2911, 1990.
- Debets, J.M.H., van der Linden, C.J., Dieteren, I.E.M., Leeuwenberg, J.F.M., Buurman, W.A. Fc-Receptor Cross-linking Induces Rapid Secretion of Tumor Necrosis Factor (Cachectin) by Human Peripheral Blood Monocytes. *The J. of Immunology.* 141(4):1197-1201, August 15, 1988.

- Delpierre, M., Panijel, J., Terquem, M. Lymphoid Cells From Senescent Animals Contain Specific Inhibitors of Immune Response. *Cellular Immunology*. 52:204-217, 1980.
- Deitch, E.A. The Management of Burns. *The N.E. J. of Med.* 323(18):1249-1253, 1990.
- Deitch, E.A. The Role of Intestinal Barrier Failure and Bacterial Translocation in the Development of Systemic Infection and Multiple Organ Failure. *Arch. Surg.* 125:403-404, March, 1990.
- Deuschle U., and Weser, U. Copper and Inflammation. *Progress in Clinical Biochemistry and Medicine, Volume 2.* Springer-Verlag Berlin Heidelberg, pp. 97-129, 1985.
- Deuster, P.A., Morrison, S.D., Ahrens, R.A. Endurance Exercise Modifies Cachexia of Tumor Growth in Rats. *Med. and Sci. in Sports and Ex.* 7(3):385-392, 1985.
- Dinarello, C.A., and Bernheim, H.A. Ability of Human Leukocytic Pyrogen to Stimulate Brain Prostaglandin Synthesis in vitro. *J. Neurochem.* 37:702-708, 1981.
- Dinarello, C.A., and Mier, J.W. Current Concepts: Lymphokines. *The N.E. J. of Med.* 317(15):940-945, October 8, 1987.
- du Moulin, G.C., Paterson, D., Hedley-Whyte, J., Broitman, S.A. E. coli Peritonitis and Bacteremia Cause Increased Blood-Brain Barrier Permeability. *Brain Res.* 340:261-268, 1985.
- Dunn, D.L., Barke R.A., Ewald, D.C., Simmons, R.L. Effects of *Escherichia coli* and *Bacteriodes fragilis* on Peritoneal Host Defenses. *Infection and Immunity.* 48(2):287-291, 1985.
- Eastgate, J.A., Symons, J.A., Wood, N.C., Grinlinton, F.M., di-Giovine, F.S., Duff, G.W. Correlation of Plasma Interleukin-1 Levels With Disease Activity in Rheumatoid Arthritis. *Lancet.* 2(8613):706-709, September 24, 1988.
- El-Maahlem, H., Fletcher, J. Effects of Surgery on Neutrophil Granulocyte Function. *Infection and Immunology*, pp. 32-38, 1981.
- Epstein, F.H. Cachectin: More Than a Tumor Necrosis Factor. *The N.E. J. of Med.* 316(7):379-385, 1987.
- Farris, E.J., and Griffith, J.Q. *The Rat in Laboratory Investigation.* 2nd Edition, J.B. Lippincott Co., pp. 411-413, 1949.

Flohé, L. and Giertz, H. Endotoxins, Arachidonic Acid, and Superoxide Formation. Reviews of Infectious Diseases. 9(suppl. 5):S553-S561, September-October, 1987.

Freudenberg, M.A., Freudenberg, N., Galanos, C. Time Course of Cellular Distribution of Endotoxin in Liver, Lungs and Kidneys of Rats. Br. J. Exp. Path. 63:65-65, 1982.

Freudenberg, M.A., Kleine, B., Freudenberg, N., Galanos, C. Distribution, Degradation and Elimination of Intravenously Applied Lipopolysaccharide in Rats. Bacterial Endotoxin Chemical, Biological Aspects. Ed. Homma, H.Y., Kanegasaki, S., Luderitz, O., Shiba, T., Westphal, O. Verlag Chemic, Weinheim, 1984.

Ganong, W.F. Review of Medical Physiology. 12th Edition. Appleton-Century Crofts, East Norwalk, CT, pp. 21, 437, and 578-579, 1985.

Ganong, B.R., Loomis, C.R., Hannun, Y.A., Bell, R.M. Regulation of Protein Kinase C by Lipid CoFactors. Cell Membranes, Methods and Reviews, Volume 3. Ed., Elson, E., Frazier, W., Glase, L. Plenum Press, N.Y., 1987.

Goldenberg, D.L., Reed, J.I. Bacterial Arthritis. The N.E. J. of Med. 312(12):764-770, March, 1985.

Goodrick, C.L., Ingram, D.K., Reynolds, M.A., Freeman, J.R., Cider, N.L. Differential Effects of Intermittent Feeding and Voluntary Exercise on Body Weight and Lifespan in Adult Rats. J. of Geront. 38(1):36-45, 1983.

Goodwin, J.S., Bromberg, S., Staszak, C., Kasubowski, P.A., Messner, R.P., and Neal, J.F. Effect of physical stress on sensitivity of lymphocytes to inhibition by Prostaglandin E2. J. Immunology. 127:518, 1981.

Goris, R.J.A. The Role of Bacteria and Endotoxins in Multiple Organ Failure. Infections in Medicine, pp. 22-33, August, 1992.

Grosh, W.W., and Quesenberry, P.J. Recombinant Human Hematopoietic Factors in the Treatment of Cytopenias. Clin. Immunology and Immunopath. 62(1):S25-S38, 1992.

Hakkert, B.C., Kuijpers, T.W. Leewenberg, J.F.M., van Mourik, J.A., Roos, D. Neutrophil and Monocyte Adherence to and Migration Across Monolayers of Cytokine-Activated Endothelial Cells: the Contribution of CD18, ELAM-1, and VLA-4. Blood. 78(10):2721-2726, 1991.

- Hawryloxicz, C.M., Howells, G.L., Feldmann, M. Platelet-derived Interleukin 1 Induces Human Endothelial Adhesion Molecule Expression and Cytokine Production. *J. of Exp. Med.* 174:785-790, 1991.
- Heggors, J.P., and Robson, M.C. Prostaglandins and Thromboxane. *Critical Care Clinics.* 1(1):59-77, March 1985.
- Hogan, M.M., Vogel, S.N. Inhibition of Macrophage Tumoricidal Activity by Glucocorticoids. *The J. of Immunology.* 140(2):513-519, January 15, 1988.
- Horan, M.A., and Brouwer, A. Gerontology: Approaches to Biomedical and Clinical Research. Edward Arnold, a division of Hudder and Stoughton, London, 1990.
- Issekutz, A.C., and Issekutz, T.B. Cellular and Vascular Phenomena in Inflammation. *Methods in Enzymology.* 162:301-319, 1988.
- Jandl, R.C., Andre-Schwartz, J., Borges-DuBois, L., Knipes, R.S., McMurrich, B.J., Babior, B.M. Termination of the Respiratory Burst in Human Neutrophils. *J. Clin. Invest.*, pp. 1176-1185, 1978.
- Johansen, K.S., Berger, E.M., Repine, J.E. Effect of Temperature on Polymorphonuclear Leukocyte Function. *Acta Path. Microbiol. Immunol. Scand. Sect. C* (1:355-359, 1983.
- Johnston, R.B., Guthrie, L.A., McPhail, L.C. Priming of Neutrophils for Enhanced Oxidative Metabolism by Bacterial Endotoxin. Oxy Radicals and Their Scavenger Systems. Vol. II: Cellular and Medical Aspects. Elsevier Science Publishing Co., Inc. Greenwald, R.A and Cohen, G., editors, 1983.
- Johnston, R.B., and Kitagawa, S. Molecular Basis for the Enhanced Respiratory Burst of Activated Macrophages. *Federation Proc.* 44(14):2927-2932, 1985.
- Kampschmidt, R.F.M Upchurch, H.F. Neutrophil Release After Injections of Endotoxin or Leukocytic Endogenous Mediator Into Rats. *J. of the Reticuloendothelial Society.* 28(2):1991-201, 1980.
- Kane, R.L., Ouslander, J.G., Abrass, I.B. Essentials of Clinical Geriatrics, 2nd Ed. McGraw-Hill Information Services Co., Health Professions Division, New York, 1989.

- Kenney, R.A., Coll, A., Harrington, M.G., Hodkinson, H.M., Pepys, M.B. A Comparison of the Erythrocyte Sedimentation Rate and Serum C-Reactive Protein Concentration in Elderly Patients. Age and Ageing. 14:15-20, 1985.
- Kitahora, T., Suzuki, K., Asakura, H., Yoshida, T., Suematsu, M., Watanabe, M., Aiso, S., Tsuchiya, M. Active oxygen Species Generated by Monocytes and Polymorphonuclear Cells in Crohn's Disease. Digestive Disease Science. 33(8):951-958, 1988.
- Klebanoff, S.J. Myeloperoxidase: Contribution to the Microbicidal Activity of Intact Leukocytes. Science. 169:1065-1067, 1970.
- Klebanoff, S.J. Oxygen-Dependent Cytotoxic Mechanisms of Phagocytes. Advances in Host Defense Mechanisms, Volume 1. Galin, J.I. and Fauci, A.S. editors, Raven Press, New York, 1982.
- Klebanoff, S.J., and Pincus, S.H. Hydrogen Peroxide Utilization in Myeloperoxidase-Deficient Leukocytes: a Possible Microbicidal Control Mechanism. J. of Clin. Invest. 50:2226-2229, 1971.
- Klempner, M.S., Dinarello, C.A., Henderson, W.R., Gallin, J.I. Stimulation of Neutrophil Oxygen-dependent Metabolism by Human Leukocytic Pyrogen. J. of Clin. Invest. 64:996-1002, 1979.
- Knook, D.L., Brouwer, A. Kupffer Cells and the Acute Phase Response: the Effect of Aging. Immunologic Invest. 18(1-4):339-351, 1989.
- Korchak, H.M., Vienne, K., Rutherford L.E., Weissmann, G. Neutrophil Stimulation: Receptor, Membrane and Metabolic Events. Federation Proc. 43(12):2749-2754, September, 1984.
- Kozma, C.K., Weisbroth, S.H., Stratman, S.L., Conjeros, M. Normal Biological Values for Long-Evans Rats. Laboratory Animal Care. 19(5):746-755.
- Kuehl, F.A., Egan, R.W. Prostaglandins, Arachadonic Acid and Inflammation. Science. 210(28):978-984, November, 1984.
- Lavie, L., and Gershon, D. Oxygen Free Radical Production by Mouse Peritoneal Macrophages as a Function of Age. Mech. of Aging and Dev. 45:177-189, 1988.

Le, J., and Vilcek, J. Biology of Disease. Tumor Necrosis Factor and Interleukin 1: Cytokines with Multiple overlapping Biological Activities. Laboratory Invest. 56(3):234-248, 1987.

Leff, R.D., Towles, W., Aldo-Benson, M.A., Madura, J., Biegel, A.A. A Prospective Analysis of the Arthritis Syndrome and Immune Function in Jejunoileal Bypass Patients. The J. of Rheum. 10(4):612-618, 1983.

Loria, A., et al. Serum Ferritin in an Elderly Population. J. of Geriatrics. 34(4):521-524, 1979.

MacKinney, A.A. Effects of Aging on the Peripheral Blood Lymphocyte Count. J. of Geront. 33(2):213-216, 1978.

Maizel, A.L., and Lachman, L.B. Biology of Disease. Control of Human Lymphocyte Proliferation by Soluble Factors. Lab. Invest. 50(4):329-377, 1984.

Massey, V., Strickland, S., Mayhew, S.G., Howell, L.G. Engel, P.C., Mathews, R.G., Schuman, M., Sullivan, P.A. The Production of Superoxide Anion Radicals in the Reaction of Reduced Flavins and Flavoproteins With Molecular Oxygen. Biochemical and Biophysical Res. Commun. 36(6):891-897, 1969.

McCarthy, D.A., et al. Morphological Evidence that Activated Polymorphs Circulate in the Peripheral Blood of Patients with Rheumatoid Arthritis. Annals of the Rheumatic Diseases. 51(1):13-18, 1992.

McCord, J.M., Boyle, J.A., Day, E.D., Rizzolo, L.J., Salin, M.L. A Manganese-containing Superoxide Dismutase From Human Liver. Superoxide and Superoxide Dismutases. Ed. by A.M. Michelson, J.M. McCord and I. Fridovich. Academic Press, London, pp. 129-138, 1977.

McCord, J.M. The Superoxide Free Radical: Its Biochemistry and Pathophysiology. Surgery. 94(3):412-414, 1983.

The Merck Manual of Geriatrics. Abrams, W.B. and Berkow, R., Editors. Merck Sharp and Dohme Research Laboratories, Merck and Co., Inc., Rahway, N.J., 1990.

Michelson, A.M. Production of Superoxide by Metal Ions. Superoxide and Superoxide Dismutases. Ed. by A.M. Michelson, J.M. McCord and I. Fridovich. Academic Press, London, pp. 78-86, 1977.

Michie, H.R., Manogue, K.R., Spriggs, D.R., Revhaug, A., O'Dwyer, S., Dinarello, C.A., Cerami, A., Wolff, S.H., and Wilmore, D.W. Detection of Circulating Tumor Necrosis Factor After Endotoxin Administration. *The N.E. J. of Med.* 318(23):1481-1486, 1988.

Miller, F.N., Sims, D.E. Contractile elements in the regulation of macromolecular permeability. *Federation Proc.* 45:84-88, 1986

Mills, J.A. Do Bacteria Cause Chronic Polyarthriti? *The N.E. J. of Med.* 320(4):245-246, 1989.

Minotti, G., Aust, S.D. The Requirement for Iron (III) in the Initiation of Lipid Peroxidation by Iron (II) and Hydrogen Peroxide. *The J. of Biol. Chem.* 262(3):1098-1104, 1987.

Moldawer, L.L., Marano, M.A., Wei, H., Fong, Y., Silen, M.L., Kuo, G., Manogue, K.R., Vlassara, H., Cohen, H., Cerami, A., Lowery, S.F. Cachectin/Tumor Necrosis Factor α Alters Red Blood Cell Kinetics and Induces Anemia in vivo. *Federation Proc.* 3:1637-1643, 1989.

Moolenaar, W.H., Tertoolen, L.G.J., de Laat, S.W. Phorbol Ester and Diacylglycerol Mimic Growth Factors in Raisin Cytoplasmic pH. *Nature.* 312(22):371-377, 1984.

Morrisca, D.C., Ryan, J.L. Bacterial Endotoxins and Host Immune Response. Advances in Immunology. Academic Press, Inc. 28:293-450, 1979.

Morrison, D.C., Vukajlovich, S.W., Goodman, S.A., Wollenweber, H.W. Regulation of Lipopolysaccharide Biological Activity by Polysaccharide. Bayer-Symposium VIII. The Pathogenesis of Bacterial Infections. Springer-Verlag Berlin Heidelberg, pp. 68-84, 1985.

Morrow, L.E., McClellan, J.L., Conn, C.A. and Kluger, M.J. Glucocorticoids Alter Fever and IL-6 responses to Psychological Stress and to Lipopolysaccharide. *Am. J. Physiol.* 264(Regulatory Integrative Comp. Physiol. 33):R1010-R1016, 1993.

Munster, A.M. Immunologic Alterations Following Injury. *Advances in Orthopedic Surgery.* William & Wilkins Co., pp. 328-331, 1985.

Ng, Y.C., Peters, D.K., Walport, M.J. Monoclonal Rheumatoid Factor-IgG Immune Complexes: poor fixation of Opsonic C4 and C3 Despite Efficient Complement Activation. *Arth. and Rheum.* 31(1):99-107, 1989.

- Ohmann, H.B., Campos, M., Lawman, M.J.P., Babiuk, L.A. Induction of MHC Class II Antigens on Bovine Cells of Nonlymphoid Origin by Recombinant Bovine Interferon- γ and Tumor Necrosis Factor- α . *J. of Interferon Res.* 8:451-462, 1988.
- Olofsson, P., Nylander, G., Olsson, P. Endotoxin: Routes of Transport in Experimental Peritonitis. *Am. J. of Surg.* 151:443-447, 1986.
- Patton, W.N., Meyer, P.J., and Stuart, J. Evaluation of sealed vacuum extraction method (Seditainer) for measurement of erythrocyte sedimentation rate. *J. Clinical Pathology*, 42:313-317, 1989.
- Paul, B.B., Strauss, R.R., Jacobs, A.A., Sbarra, A.J. Function of H_2O_2 , Myeloperoxidase, and Hexose Monophosphate Shunt Enzymes in Phagocytizing Cells From Different Species. *Infection and Immunity.* 1(4):338-344, 1970.
- Peichl, P., Ceska, M., Broell, H., Effenberger, F., Lindley, I.J.D. Human Neutrophil Activating Peptide/Interleukin 8 Acts as an Autoantigen in Rheumatoid Arthritis. *Annals of the Rheumatic Diseases.* 51(1):19-22, 1992.
- Raetz, C.R.H., Ulevitch, R.J., Wright, S.D., Sibley, C.H., Ding, A., Nathan, C.F. Gram-Negative Endotoxin: an Extraordinary Lipid With Profound Effects on Eukaryotic Signal Transduction. *The FASEB Journal.* 5:2652-2660, 1991.
- Reinehart J.J., Balcerzak, S.P., Sagone, A.L., LoBuglio A.F. Effects of Corticosteroids on Human Monocyte Function. *The J. of Clin. Invest.* 54:1337-1343, December, 1974.
- Reinhart, W.H. Blood Sedimentation—a Simple and Useful Test? (Abstract only, translated from the German language) *Schweiz Med Wochenschr* 118(22):839-844, June 4, 1988.
- Rikans, L.E., and Moore, D.R. Effect of Aging on Aqueous-phase Antioxidants in Tissues of Male Fischer Rats. *Biochimica et Biophysica Acta.* 966:269-275, 1988.
- Rose, N.R., and Bona C. Defining Criteria for Autoimmune Disease (Witebsky's postulates revisited). *Immunology Today.* 14(9):426-429, 1993.

- Ryan, G.B., and Majno, G. Inflammation. The UpJohn Company, Kalamazoo, Michigan, 1977, pp. 7-77.
- Sagone, A.L., King, G.W., Metz, E.N. A Comparison of the Metabolic Response to Phagocytosis in Human Granulocytes and Monocytes. *The J. of Clin. Invest.* 57:1352-1258, May, 1976.
- Salin, M.L., and McCord, J.M. Free Radicals in Leukocyte Metabolism and Inflammation. Superoxide and Superoxide Dismutases. Michelson, A.M., McCord J.M., and Fridovich I., Editors. Academic Press, London, pp. 258-270, 1977.
- Samuni, A., Black, C.D.V., Krishna, C.M., Malech, H.L., Bernstein, E.F., Russo, A. Hydroxyl Radical Production by Stimulated Neutrophils Reappraised. *The J. of Biol. Chem.* 263(27):13797-13891, September 25, 1988.
- Sandvig, K. and van Deurs, B. Endocytosis Without Clathrin. *Trends in Cell Biol.* 4:275-277, August, 1994.
- Sasada, M., Pabst, M.J., Johnston, R.B. Activation of Mouse Peritoneal Macrophages By Lipopolysaccharide Alters the Kinetic Parameters of the Superoxide-producing NADPH Oxidase. *The J. of Biol. Chem.* 258(16):9631-9635, 1983.
- Savill, J.S., Wyllie, A.H., Henson, J.E., Walport, M.J., Henson, P.M., Haslett, C. Macrophage Phagocytosis of Aging Neutrophils in Inflammation Programmed Cell Death in the Neutrophil Leads to its Recognition by Macrophages. *J. Clin. Invest.* 83:865-875, March, 1989.
- Schaffner, A. Schaffner, T. Glucocorticoid-Induced Impairment of Macrophage Antimicrobial Activity: Mechanisms and Dependence on the State of Activation. *Reviews of Infectious Diseases.* 9(suppl. 5):S620-S629, September-October, 1987.
- Schumann, R.R., Leong, S.R., Flaggs, G.W., Gray, P.W., Wright, S.D., Mathison, J.C. Tobias, P.S., Ulevitch, R.J. Structure and Function of Lipopolysaccharide Binding Protein. *Science.* 249:1429-1433, 1990.
- Schutze, S., Berkovic, D., Tomsing, O., Unger, C., Krönke, M. Tumor Necrosis Factor Induces Rapid Production of 1' 2' Diacylglycerol by a Phosphatidylcholine-specific Phospholipase C. *J. of Exp. Med.* 174:975-988, 1991.
- Shamburek, R.D., and Farrar, J.T. Disorders of the Digestive System in the Elderly. *The N.E. J. of Med.* 322(7):438-443, February 15, 1990.

- Shepro, D. The microvascular system. Cell Biology of Inflammation (Handbook of Inflammation, v.2). Weissmann, G., editor. Elsevier/North-Holland Biomed. Pr., New York, pp. 27-51, 1980.
- Siegel, J. Demographic Aspects of Aging and the Older Population in the United States, Current Population Reports, Series P-23, No. 59, 1976.
- Skau, T., Tenger, Y. Spontaneous Peritonitis and Rheumatoid Arthritis - a Case Report. Acta. Chir. Scand. 152:317-318, 1986.
- Skau, T., Nyström, P-O., Öhman, L., Stendahl, O. The Kinetics of Peritoneal Clearance of *Escherichia coli* and *Bacterioides fragilis* and Participating Defense Mechanisms. Arch. Surg. 121:1033-1039, September, 1986.
- Smith, G.H. The Bactericidal Action of the Serum Following Injections of Adrenalin. The J. of Immunology, 12(3):205-218, 1926.
- Smith, R.J., Epps, D.E., Justen, J.M., Sam, L.M. Wynalda, M.A., Fitzpatrick, F.A., Yein, F.S. Human Neutrophil Activation with Interleukin-1: a Role for Intracellular Calcium and Arachidonic Acid Lipoxygenation. Biochem. Pharm. 36(22):3851-3858, 1987.
- Smith R.M., and Mohideen, P. One Hour in 1 ATA Oxygen Enhances Rat Alveolar Macrophage Chemiluminescence and Fungal Cytotoxicity. Am. J. Physiol. 260(Lung Cell. Mol. Physiol. 4):L457-L463, 1991.
- Southorn, P.A., Powis, D.G. Free Radicals in Medicine. I. Chemical Nature and Biologic Reactions. Mayo Clinic Proceedings. 63:381-389, 1988.
- Southorn, P.A., Powis, D.G. Free Radicals in Medicine. II. Involvement in Human Disease. Mayo Clinic Proceedings. 63:390-408, 1988.
- Tanamoto, K., Shade, U., Rietschel, E.T., Kusumoto, S., and Shiba, T. Endotoxic Induction of Prostaglandin Release from Macrophages by Nontoxic Lipid A Analogs Synthesized Chemically. Infection and Immunity, 58(1):217-221, January, 1990.
- Thelen, M., Dewald, B., Baggiolini, M. Neutrophil Signal Transduction and Activation of the Respiratory Burst. Physiological Reviews. 73(4):797-821, October 1993.

- Thornhill, M.H., Kyan-Aung, U., Lee, T.H., Haskard, D.O. T Cells and Neutrophils Exhibit Differential Adhesion to Cytokine-stimulated Endothelial Cells. *Immunology*. 69:287-292, 1990.
- Tranquillo, R.T., Zigmond, S.H., Lauffenburger, D.A. Measurement of the Chemotaxis Coefficient for Human Neutrophils in the Under-Agarose Migration Assay. *Cell Motility and Cytoskeleton*. 11:1-15, 1988.
- Uauy, R., Winterer, J.C., Bilmazes, C., Haverberg, L.N. Scrimshaw, N.S., Munro, H.N., Young V.R. The Changing Pattern of Whole Body Protein Metabolism in Aging Humans. *J. of Geront.*, 33(5):663-671, 1978.
- Ulich, T.R., del Castillo, J., Ni R-X., Bikhazi, N., Calvin, L. Mechanisms of Tumor Necrosis Factor Alpha-Induced Lymphopenia, Neutropenia, and Biphasic Neutrophilia: A Study of Lymphocyte Recirculation and Hematologic Interactions of TNF- α With Endogenous Mediators of Leukocyte Trafficking. *J. Leukocyte Biol.* 45:155-167, 1989.
- Uysal, M., Seckin, S., Kocak-Toker, N., and Öz, H. Increased Hepatic Lipid Peroxidation in aged Mice. *Mech. of Ageing and Dev.* 48:85-89, 1989.
- van der Poll, T., Büller, H.R., ten Cate, H., Wortel, C.H., Bauer, K.A., van Deventer, S.J.H., Hack, C.E., Saurwein, H.P., Rosenberg, R.D., ten Cate, J.W. Activation of Coagulation After Administration of Tumor Necrosis Factor to Normal Subjects. *The N.E. J. of Med.* 332(23):1522-1627, June 7, 1990.
- von Zglinicki, T. A Mitochondrial Membrane Hypothesis of Aging. *J. of Theoretical Biology.* 127:127-132, 1987.
- Wake, K., Decker, K., Kirn, K., Knook, D.L., McCuskey, R.S., Bouwens, L., and Wisse, E. Cell Biology and Kinetics of Kupffer Cells in the Liver. *International Review of Cytology*, Vol. 118, pp. 173-229, 1989.
- Warren, J.S. Inflammation. *Trends in Biomed. Res.* 6(7):450-459, September, 1993.
- Weiss, S.J. Tissue Destruction by Neutrophils. *The N.E. J. of Med.* 320(6): 365-376, February 9, 1989.
- Wright, S.D., Jong, M.T.C. Adhesion-Promoting Receptors on Human Macrophages Recognize *Escherichia Coli* by Binding to Lipopolysaccharide. *J. of Exp. Med.* 164:1876-1888, 1986.

Ziegler, E.J. Tumor Necrosis Factor in Humans. The N.E.
J. of Med. 318(23):1533-1534, June 9, 1988.