ASPECTS OF THE INGESTIVE AND CYTOPEPTIC ACTION OF PHAGOCYTES

by

Ko Ko Gyi

A thesis submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Bacteriology

University of Utah

March, 1957

This Thesis for the Ph. D. degree

by

Ko Ko Gyi

has been approved by

The author wishes to express his appreciation to Dr. Stanley Marcus for this guidance and encouragement, and Dr. Louis P. Gebhardt, Dr. Paul S. Nicholes, Dr. John G. Bachtold and Mr. Douglas W. Hill for their suggestions and criticisms.

The research reported herein was supported under contract AF 18(600)946 with the U.S.A.F. School of Aviation Medicine, Randolph Field, Texas.

TABLE OF CONTENTS

TABLES AND FIGURES

Tables

Figures

ASPECTS OF THE INGESTIVE AND CYTOPEPTIC ACTION OF PHAGOCYTES

I. Introduction

The normal mammalian host is equipped with a complex system of humoral and cellular defenses for combatting invading microorganisms or their toxic products. Chemotaxis, cytopeptic action of phagocytes (intracellular digestion), inflammation, antibody, bactericidal action of body fluids, and anatomical and physiological barriers are among the most significant host defense mechanisms currently recognized. Knowledge concerning the extent to which each of these so-called host defenses participates in resistance to infections is incomplete. The existence of interrelationships among these processes, as well as the number of infectious agents to which the host is naturally resistant or susceptible, makes difficult evaluation of the specific role of each of these defense systems in infection and immunity. Moreover, the mechanisms by which these host defenses destroy microorganisms remain obscure.

The ability of certain cells of higher animals to ingest and digest foreign particles has been observed by many investigators since the early days of cellular pathology, (Langhans, 1870; Panum, 1874; Roser, 1881) but it was not until 1882 that Metchnikoff (Metchnikoff, 1905) first

suggested the importance of these cells in inflammatory processes and resistance of the mammalian host. He classified the fixed phagocytes which were found in the liver, spleen and lymph nodes and the large free mononuclear phagocytes of the circulating blood as "macrophages"; he called the neutrophilic leucocytes of the blood "microphages". Aschoff (1924) grouped the fixed phagocytes, including the fixed macrophages of Metchnikoff, into the reticulo-endothelial system (RES). Since the classical observations of phagocytosis by Metchnikoff, extensive studies pertaining to the precise mechanisms of this process have been made (Fenn, 1922; Jaffe, 1931; Mudd et al., 1934; Berry and Spies, 1949; Wright and Dodd, 1955). The process of phagocytosis can be conveniently divided into three stages: (1) the movement of phagocytes toward the site of invasion by infectious agent (chemotaxis); (2) the process of engulfing (phagocytosis) and, (3) the actual destruction of the ingested foreign material (intracellular digestion or cytopeptic action). The phagocytic cells by virtue of being endowed with ingestive and digestive potentialities, are therefore of utmost importance in combatting infections as well as in eliminating foreign matter from the body. Furthermore, these cells are also involved in the production of antibodies (Burnet and Fenner, 1949).

In the present investigation, studies have been made of phagocytic and cytopeptic action by phagocytes. X-irradiation has been employed for its known capacity to reduce resistance

to infection. Immunization has been employed to enhance the specific resistance mechanism (s). Also, combinations of these procedures have been employed with the hope of gaining insight into the role of cellular immunity in resistance to infection.

REVIEW OF THE LITERATURE

II. Phagocytosis

According to current concepts the phagocytic system represents a functional unit composed of widely dispersed and morphologically distinct cell types. It consists of (1) polymorphonuclear and mononuclear leucocytes of the circulation, and (2) the so-called reticulo-endothelial system (RES) in which are included the monocytes of the circulating blood, sessile cells of the endothelium lining the sinusoids of liver, spleen, bone marrow, lymph nodes, adrenal cortex and anterior lobe of the pituitary, sessile or resting-wandering cells ("histiocytes") of the connective tissues in various organs, microglial cells and alveolar septal cells of the lung (Downey, 1938; Suter, 1956). A diagramatic representation of the phagocytic system is given in Table 1. The evidence indicating the importance of phagocytes in resistance to infectious disease was summarized by Bordet (1939) as follows:

1. A parallelism exists between the efficiency of phagocytosis and resistance to infection.

2. The bactericidal power attributable to the humoral factors of the blood is not enough to destroy pathogenic microorganisms. It is the intracellular digestion following uptake by phagocytes that causes destruction of the parasites.

3. The virulence of microorganisms is often associated with antiphagocytic properties.

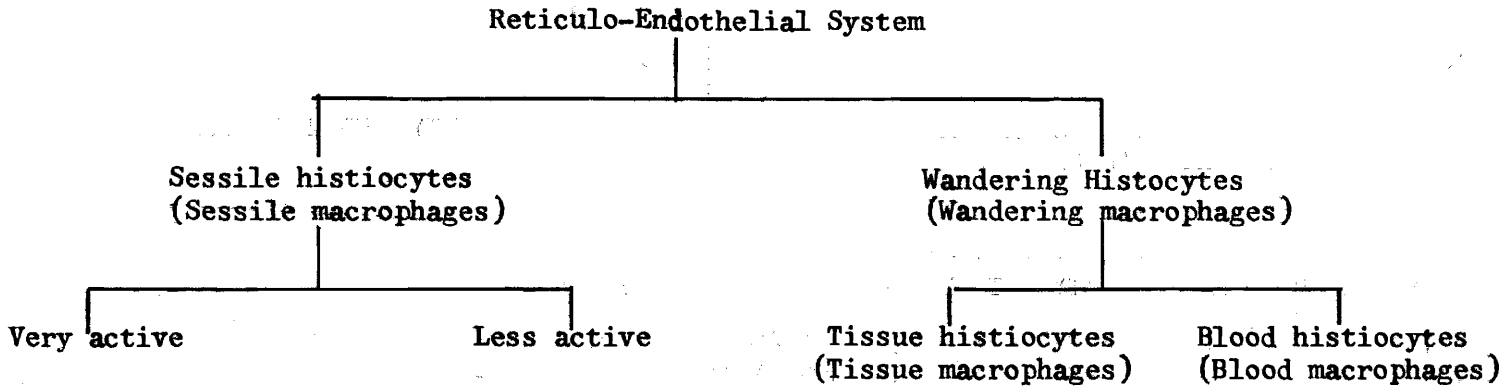
4. The sequence of the disease process is less serious when inoculation of organisms is made at a site abundant in phagocytes.

5. All the factors that interfere with phagocytosis diminish the resistance of animals to infection.

6. The major significance of phagocytosis is shown by the fact that a favorable course in a disease process is usually accompanied by an increase in the number of leucocytes. In the immunized animal, following infection leucocytosis is pronounced and is maintained for a longer period.

A. The role of the reticulo-endothelial system in the removal of colloidal particles, bacteria or viruses from the circulation. Studies of the removal of foreign particles from the blood stream of animals date to the pre-bacteriological era where Hoffmann and von Recklinghauser (1867) and Ponfick (1869) noted that particles of carmine and vermillion injected into animals were not eliminated in the urine or bile but were deposited in various organs such as the spleen, liver and the lymph nodes. These particles remained recognizable at these sites for weeks. Wyssokowitch (1886) injected intravenously large numbers of many species of bacteria into rabbits and dogs and showed that both the pathogenic and non-pathogenic organisms disappeared from the blood stream in four hours. However, the pathogenic organisms reappeared

TABLE I.


Reticulo-Endothelial System
Sessile histiocytes
(Sessile macrophages)
Wandering Histocytes
(Wandering macrophages)
Very active
Less active
Tissue histiocytes
(Tissue macrophages)
Blood histiocytes
(Blood macrophages)
Endothelium of:
Liver capillaries,
Spleen sinuses,
Lymph sinuses,
Marrow sinuses,
Lung capillaries,
Adrenal capillaries,
Pituitary capillaries.
Reticular cells of:
Spleen
Lymphatic glands and
tissues
Thymus.

after some hours and progressively increased in numbers until the animals succumbed. Many of the bacteria injected were found to be concentrated in the spleen and liver. Subsequent studies (Bull, 1915; Manwaring and Coe, 1916; Hopkins and Parker, 1918; Sullivan et al., 1934; Reichel, 1939, Bennett and Beeson, 1954.) on this phase of the mechanisms of removal of microorganisms from the blood stream have confirmed the earlier findings. It appears from the later studies cited above that the clearance rate of bacteria from the blood stream as well as from other tissues is dependent upon the virulence of the invading organisms for the host under consideration as well as the hosts' natural resistance or induced immunity. The relationship of immunization to phagocytic activity will be discussed in section V.

Investigations concerning the blood clearing mechanism employing different inorganic colloids have been reported. Harrington and Huggins (1939) studied the rate of disappearance from the blood stream of intravenously injected thorium dioxide in normal dogs. They found that the colloid was removed rapidly during the first twenty minutes after injection and then at a slower rate until it could no longer be detected. This removal took place in ten to twelve hours. Dobson et al., (1949) noted that the rate of removal and organ of deposition depended on the size of colloids injected. They reported that colloids of larger magnitude such as zirconium showed rapid disappearance from the circulation and

were deposited mainly in the phagocytes of the liver and spleen. Colloids of smaller size such as yttrium disappeared comparatively more slowly than the larger colloids and were primarily deposited in the bone marrow and spleen, and secondarily in the liver. Jallut and his associates (1955), confirming these findings reported that a suspension of large particles of $CrPO_4$ (average diameter 1.3u), when injected intravenously into mice, was deposited mostly in the liver, and to a lesser extent in the lungs and in the spleen. However, in the case of a suspension of finer particles (average diameter 0.008u) deposition occurred mostly in the liver, less in the lungs and only insignificantly in the spleen. These workers also noted that the rate of disappearance from the blood did not depend on the diameter but on the number of injected particles. Localization in the organs however, depends on the diameter of the injected particles. Studies conducted by different investigators employing a variety of colloids including colloidal gold (Sheppard et al., 1951), colloidal zirconium and yttrium (Dobson et al., 1949), colloidal iron saccharate (Nissim, 1953) and carbon particles (Halpern et al., 1951) have indicated that the rate of disappearance from the blood stream of intravenously injected colloid can be expressed as an exponential function.

The reticulo-endothelial system has also been demonstrated to be capable of removing not only bacteria and colloidal particles but also other microorganisms such as

viruses, fungi and protozoa (Taliaferro, 1949; Nungester, 1954). Nungester and Watrous (1939) found that following intravenous injection of bacteriophage these organisms could be found deposited in the liver and spleen. Comparative studies employing staphylococci showed that these organs accumulated the organisms to the same degree that they did phage. Phagocytosis of herpes simplex virus by rabbit macrophages in vitro have been described by Garabedian and Syverton (1954). They suggested that phagocytosis may play a role in the dissemination or destruction of the virus in this infection. Bubel and Wilcox (1957) carried out in vitro studies of the phagocytosis of poliomyelitis virus by peritoneal leucocytes obtained from monkeys. The technique used consisted of first mixing virus and the cells and then determining the rate of disappearance of virus from the medium. These workers found that virus concentration in the medium was reduced to half in 30 minutes. Moreover, titration of virus concentration in the phagocytes at different intervals suggested that inactivation of virus had taken place. Whether the removal of phage from the blood stream by the spleen and liver phagocytes or of virus particles, by exudate leucocytes as observed by these latter investigators involve mechanisms similar to those that have been observed for other microscopic particles cannot be stated with assurance at the present time. Further work, to differentiate this mechanism of phagocytosis from commonly recognized virus-host cell interaction, is necessary before

the role and significance of phagocytosis in viral infection can be established.

B. The role of the polymorphonuclear leucocyte in the disposal of microorganisms from the circulation. Although most of the bacteria or inert particles which are removed from the circulation following intravenous injection are found in the liver or spleen, an appreciable number of organisms are found in the lungs. When pathogenic or nonpathogenic bacteria are implanted in the air sacs in considerable numbers there occurs a migration of fluid and cells from the blood stream into the alveolar spaces. The initial cellular response to all the common types of bacterial pulmonary invasion (pneumococci, streptococci, staphylococci and B. friedlanderi) is predominantly neutrophilic in character (Robertson, 1941). Histologically many of these organisms are found to be ingested by polymorphonuclear leucocytes within the alveolar capillaries. The role of the neutrophilic leucocytes in the reaction to pulmonary pneumococus infection appears to be thoroughly established. This has been described by Robertson et al., (1933) in experimental canine pneumonia and by Gunn and Nungester (1936) in experimental pneumonia in rats. These investigators noted that polymorphonuclear leucocytes accumulated early at the site of the implanted microorganisms and began to phagocytise pneumococci within a few hours. As the intensity of the cellular mobilization increased, more of the bacteria were engulfed and in the central parts of

the lesion they seemed to be in the process of digestion within the leucocytes. Wood et al., (1951) made observations of the intravascular reaction to injection of pneumococci and of Friedlander's bacilli in the rabbit ear chamber. These workers found that immediately after injection, the polymorphonuclear leucocytes were seen to stick to the endothelium of capillaries and to become actively motile, migrating freely about the endothelial surfaces. Within fifteen minutes after injection, ingestion of bacteria by these cells began to occur by the mechanism which Wood has called "surface phagocytosis", a process which takes place in the absence of specific antibody. Kerby and Martin (1951) demonstrated that the disappearance rate of Staphylococcus aureus from the blood stream of rabbits following destruction of the polymorphonuclear leucocytes by benzol or mechlorethamine hydrochloride remained constant. In the light of these observations Wood et al., (1951) suggested that "the circulating leucocytes and reticuloendothelial cells appear to supplement one another in destroying the bacteria that have gained access to the circulation and that impaired function of one system is compensated by the other mechanism".

III. Intracellular Digestion by Phagocytes

The final stage in the process of phagocytosis is the destruction and elimination of ingested organisms or other particulate matter. From the point of view of efficient

host resistance, this step is the most crucial of all, for if the phagocytes fail to destroy the pathogens, the latter may multiply within the phagocytes with the result that a disseminating infection ensues. Phagocytes may not destroy pathogens either because their digestive potentialities are impaired (Donaldson et al., 1954) (as in the radiation syndrome) or the microorganisms resist destruction by virtue of being equipped with certain chemical substances which are relatively insusceptible to digestion (Dubos, 1945) (tubercle bacilli, encapsulated pneumococci). To date very little effort has been devoted to the study of this phase of phagocyte function. Approaches to this problem have generally been carried out by study of the enzyme content of phagocytes, changes in enzyme content in health and disease, bactericidal action of leucocytic extracts, changes in intracellular digestion following treatment with certain agents and other biochemical changes taking place in the milieu interieur of the phagocyte.

A considerable literature exists concerning the bactericidal actionof leucocytic extracts. Denys and Kaisin (1893) found that the pleural exudate of rabbits obtained by injection of dead staphylococci and freed of cells by centrifugation was bactericidal for living organisms of the same species. Subsequent studies by other investigators confirmed these observations (Schattenfroli, 1897; Pettersson, 1905). Hiss and Zinsser (1908) obtained protective results when extracts

from normal rabbit leucocytes were administered to experimental animals infected with a variety of pathogens, such as pneumococci, streptococci, meningococci, and typhoid bacilli. Similar observations were made in human beings suffering from meningitis, pneumonia and staphylococcal infections following the infusion of normal rabbit leucocytic extracts. In addition, these investigators demonstrated that leucocytic extracts did not favor phagocytosis and that their moderate bactericidal activity could not entirely account for their salutary effect on disease. Hiss and Zinsser attributed this observation to "the obscure factors which account for not infrequent successes of so-called non-specific protein therapy, which consists of the injection of almost any bacterial or other protein".

The presence of enzymes in macrophages has been recognized since Metchnikoff's first observation of intracellular digestion by phagocytes (Metchnikoff, 1905). In fact he named those leucocytic enzymes "cytases" or "digestive ferments". He found these were optimally active in a weakly acid medium. Later work by other investigators revealed the presence of a variety of enzymes in phagocytes. Opie (1922) reported finding proteolytic enzymes in leucocytes and monocytes which he called leucoprotease and lymphoprotease. Weiss and Czarnetzky (1935) found proteinases similar to pepsin and trypsin in monocytes and neutrophils obtained from rabbits. However, little evidence existed to

demonstrate that these enzymes were capable of killing bacteria. Jochmann (1912) obtained extracts of leucocytes which were actively proteolytic but found that these extracts possessed no bactericidal properties. He suggested that they may take part in the killing of bacteria provided other factors are present. No detectable bactericidal substance against B. subtilis was found in extracts of neutrophils or monocytes obtained from rabbits (Myrvik and Weiser, 1955). Salton (1953) reported that the untreated cells of certain Gram negative and Gram positive bacteria were resistant to lysis in vitro by trypsin.

By contrast with the lack of any convincing evidence in favor of destruction of bacteria by enzymes present in phagocytes, the bactericidal and lytic effect of lysozyme on a number of bacteria has been well documented (Dubos, 1954). Lysozyme has been found to be present in granulocytes (Fleming, 1922). However, this cannot be the only factor involved in bacterial destruction since this enzyme is effective only against some organisms. In this connection it is of interest to note the observations made by Grogg and Pearse (1954) who studied a variety of enzymes present in the reticulo-endothelial organs of mice, rats and guinea pigs in an attempt to correlate enzymic activity with resistance to tuberculosis. They found that large amounts of acid phosphatase were present in mononuclear phagocytes of resistant animals (mouse, rat). These investigators

therefore suggested that in such animals the phosphatides of tubercle bacilli might be employed as a substrate for the acid phosphatase and that the enzymic armament of these cells probably is a contributing factor in the mechanism of natural resistance to tuberculosis.

From the observations that death of certain bacteria within the phagocytes was of immediate nature and that certain enzymes are capable of decomposing only a portion of bacteria not essential for viability, Dubos (1954) offered a hypothesis relating the possible role of changes in the acidity of phagocytes immediately following phagocytosis with intracellular destruction of bacteria. It has been found that phagocytosis of bacteria is followed by a rise in acidity inside the phagocytes. This was deduced by Metchnikoff, as well as by other investigators, from observations of changes in color of litmus particles or dyes within phagocytic cells (Metchnikoff, 1905; Rous, 1925). From <u>in vitro</u> studies of a number of organic acids such as lactic, pyruvic, and glutamic, Dubos (1953) found that lactic acid is the most bactericidal. Since this acid has been reported to be a product of metabolism of phagocytic cells he suggested that this acid may play a role in the cause of death of ingested bacteria. In this connection it is of interest to note that enzymes that have been found to be present in the phagocytes have an optimum pH of 3 to 4 (Opie, 1922; Weiss and Czarnetzky, 1935).

Recently Hirsh (1956a) reported a substance in extracts of leucocytes as well as in extracts of organs of the RES which has bactericidal effects on various species of bacteria. He named this substance phagocytin. Phagocytin was found to be active against Gram negative bacteria and was obtainable with relative ease from rabbit leucocytes but could not be obtained from mouse or rat leucocytes. This seems to indicate that phagocytin may play a role in the destruction of bacteria by rabbit phagocytes but other substances have to be sought for in the case of cells from rats or mice. Moreover, it is of interest to note that studies (Hirsh, 1956b) involving quantitative relationships between the number of bacteria, the concentrations of phagocytin required to kill them, and the time-temperature characteristics of the lethal action of phagocytin suggest an enzymatic nature for the bactericidal reaction.

Exceptions concerning their activity have been noted then, in the case of all enzyme or enzyme-like substances, relative to their overall bactericidal activity. It is apparent that at present no one mechanism can be considered to be responsible for initiating or completing the process of killing of bacteria. Nevertheless, the occurrence of antibacterial enzymes in phagocytes, the bactericidal action of leucocytic extracts including phagocytin and changes in leucocytic acidity following phagocytosis suggest the plausibility of the hypothesis that all these processes act in

conjunction with one another in the intracellular disposal of microorganisms.

IV. Effect of X-Irradiation on Phagocyte Function

The observations that bacteremia develops following x-irradiation (Warren and Whipple, 1923; LeRoy, 1947; Bower, 1951; Miller et al., 1951) and that x-irradiated animals exhibit increased susceptibility to various types of naturally occuring or experimentally induced infections (Chrom, 1935; Miller and Hammond, 1950; Osborne et al., 1951) has been well documented. This postirradiation enhanced susceptibility to infection has been recognized as one of the major causes of death among animals exposed to ionizing radiation in the near lethal to midlethal range (Talmage, 1955; Raffel, 1956). The cause of the observed increase in susceptibility is attributed to the deleterious effect of x-irradiation on specific host defense mechanisms (Taliaferro and Taliaferro, 1951). It is not the result of any potentiating effect of x-irradiation on the pathogenicity of the microorganisms (Taliaferro and Taliaferro, 1951). Conclusive evidence is available concerning the postirradiation depression of host defenses such as the antibody forming mechanism (Hektoen, 1915; Kohn, 1951; Taliaferro and Taliaferro, 1951), and the normal bactericidal action of serum (Marcus and Donaldson, 1953; Fishman and Shechmeister, 1955). Information concerning the effect of

x-irradiation on phagocytosis however is contradictory. Furthermore, little work has been carried out relating to effects of x-irradiation on digestive function of phagocytes.

A. Effect of x-irradiation on the reticulo-endothelial system. From studies of the morphologic changes of reticulo-endothelial cells following x-irradiation, Bloom (1948) reported that they are highly resistant to x-irradiation. Similar conclusions were drawn by Brecher et al. (1948) who found no evidence of diminution or proliferation of the reticulo-endothelial cells following x-irradiation.

Studies on the effect of x-irradiation on the phagocytic function of the reticulo-endothelial system have been performed by determining the deposition of intravenously injected colloidal particles in the reticulo-endothelial organs, or the rate of disappearance of such particles from the blood stream. As early as 1935, Chrom (Chrom, 1935) observed that following the intravenous injection of living salmonella into mice, the heart blood of x-irradiated animals did not become sterile in 24 to 72 hours whereas the blood of the normal animals similarly injected was sterile within 12 to 15 hours. The blood stream of mice whose livers and spleens were protected by lead shielding during x-irradiation became sterile within 16 hours after challenge. This investigator concluded that the reticulo-endothelial cells were injured by x-irradiation. Observations of similar nature have been made by Gordon et al., (1955) who studied the rate

of removal of intravenously injected Klebsiella pneumoniae in x-irradiated rabbits (800 r) during a 2 to 10 day post-irradiation period. They noted that during the first hour or two after injection the number of bacteria in the blood of the x-irradiated rabbits decreased as rapidly as in the controls. After 8 hours however, the bacteria in the blood of the x-irradiated rabbits increased until the animals died. Such an increase in the number of organisms was not seen in the blood of the control animals. From these observations, Gordon et al., (1955) suggested that x-irradiated rabbits are able to remove large numbers of bacteria from the circulating blood as rapidly as normal rabbits but are unable to prevent their reentry into the blood stream.

A series of investigations in which radioactive materials were employed to study phagocytic activity of the reticulo-endothelial system have been reported. Fitch et al., (1953) studied the distribution of radioactivity in various organs of rats and the rates of disappearance from the blood stream of intravenously injected I-131 labeled dead typhoid organisms. They were unable to demonstrate any significant differences in either disappearance rates or organ distribution between normal and x-irradiated rats (500 r). These results confirmed the prior report of Barrow et al., (1951) who determined the 50% disappearance rate of intravenously injected radioactive colloidal gold from the blood stream of both normal and x-irradiated rabbits (800 r).

The average time required for 50% of the radioactive gold to disappear from the blood stream of normal animals was 1.41 minutes. These workers were unable to demonstrate any significant variation from this time in x-irradiated animals. Similar conclusions have been reached by other investigators. Esplin, Marcus and Donaldson (1953) reported no significant difference in the 72 hour uptake of colloidal ThO_2 by the mouse spleen following total body exposure of 100 r to 600 r. Ingraham (1955) reported that x-irradiation of rabbits in doses of 500 - 600 r failed to alter the rate or extent of removal of S-35 labeled sheep erythrocyte stromata from the circulation when tested 1 and 3 days postirradiation. In addition, the retention of radioactivity in the liver, spleen, kidney, and bone marrow was unaffected.

In contrast to the above investigators, Taplin et al., (1954) who studied the effect of roentgen irradiation on the reticulo-endothelial system in rabbits using the blood retention of intravenously injected prodigiosin as an index of phagocyte function, reported that no significant changes occurred following exposure to 300 r (LD_0) of x-irradiation. However, exposure to doses of 800 r to 1200 r (LD_{50}-LD_{100}) of whole body x-irradiation was followed by increased blood retention of the dye which appeared to be a function of the postirradiation time. In agreement with these findings, Di Luzio (1955) found depression in the uptake by rat liver of intravenously injected colloidal gold following x-irra-

diation in a dose of 1040 r (LD_{100}) when tested 4 days after x-irradiation.

Gabrieli and Auskaps (1953) studied the influence of whole body irradiation on the reticulo-endothelial system as determined by the disappearance rate of intravenously injected colloidal $CrP320_4$ in rats. They reported that from 1 to 28 days following doses of from 50 to 100 r that there is no deviation in the biological half-life (the time required for one-half of the radioactivity to disappear from the blood stream). When reticulo-endothelial blockade was induced with 0.1 ml of Thorotrast these workers obtained longer biological half-life values for the x-irradiated animals. From this observation these workers concluded that phagocytic capacity was diminished following x-irradiation. In this connection it is of interest to note observations made by Gabrieli and Cutler (1954) concerning internal beta-irradiation effects on the reticulo-endothelial system following intravenous injection of $CrP-320_4$. After injecting graded amounts of the radio-active material into rats they determined the amounts deposited in the liver. It was found that with high doses of P-32 (17 - 19 uc) that the liver ceased to accumulate radioactivity. These workers interpreted this as an indication of the destruction of the reticulo-endothelial cells by internal beta-irradiation.

In contrast to the findings of previous investigators, Wish et al., (1952) reported that one day after x-irradiation,

labeled homologous and heterologous plasma, homologous and heterologous erythrocytes, Evans blue and radioactive gold disappeared faster from the circulation of roentgen irradiated animals than from that of normal animals. These workers suggested that this difference was due to heightened capillary permeability caused by x-irradiation. Experiments involving the use of colloidal gold (Au-198) to study the rate of removal at 1, 3, 5 and 8 postirradiation days indicated that the ratio of isotope in the blood (normal/x-irradiated animals) ranged from 1.1 to 2.0 when measured 5 minutes after the injection of radioactive gold. When measured at 20 minutes after the injection the ratios were between 3.2 and 8.7.

It is apparent from the material reviewed in this section that different investigators obtained varying results regarding the effects of x-irradiation on the phagocytic activity of the reticulo-endothelial system. It is possible that these differences resulted from the variation in the colloidal particles employed for studying phagocytosis, doses of x-irradiation employed, days after x-irradiation that the tests were carried out and the species of animals employed.

B. Effect of x-irradiation on the circulating leucocytes. Knott and Watt (1929) studied the in vitro effect of x-irradiation on the blood cells. They irradiated the blood samples from normal and leukemic patients in a paraffin wax chamber. Samples of blood were taken at 5 or 10 minute

intervals during x-irradiation and the opsonic index determined using staphylococci as test organisms. These workers observed a decrease in phagocytic activity following x-irradiation in the leukocytes of both normal and leukemic blood within 35 minutes. On the other hand Glenn (1946a, 1946b) reported that the opsonocytophagic index of the leukocytes in the blood of rabbits can be increased by local treatment with x-rays. He found that this effect was most marked when a 100 r dose was used. The maximal increase in the opsonic index occurred 48 hours after x-ray treatment.

Esplin, Marcus and Donaldson (1953) employed an *in vivo* technic and noted that the percentage of active peritoneal phagocytes was increased in mice on the second and sixth postirradiation day. These investigators suggested that this could be due to the reduced leukocyte response in the x-irradiated animal which increased the percent of active phagocytes by increasing the number of collisions of bacteria with a given phagocytic cell. They noted that the smears of peritoneal fluid obtained from x-irradiated mice consistently showed more free bacteria than those obtained from normal mice, suggesting that the total phagocytosis was less in the x-irradiated animals. Similar observations were made by Fishman *et al.*, (1953) who studied the role of leukocytes in the increased susceptibility to infection of x-irradiated rats. These investigators determined the activity of phagocytes from blood samples of normal and

x-irradiated rats (600 r) at various postirradiation periods using Staphylococcus aureus as a test organism. A higher phagocytic index than normal was noted between 2 and 12 hours postirradiation. This was followed by a below-normal phagocytic index beginning on the third and extending to the eleventh postirradiation day.

Some support of previous findings concerning circulating leucocytes was provided by Wilkinson (1954) who studied the effect of total body x-irradiation (550 r) on rats at various intervals employing an in vitro technic. This investigator noted that on the first, fifth, and sixth days following x-irradiation, the percent of phagocytic neutrophils and the number of ingested bacteria per active phagocyte was higher in the blood of irradiated animals. However, from the seventh day until the thirteenth day he found a statistically significant decrease in the ability of the leukocytes to carry on phagocytosis. Fishman and Shechmeister (1955) however, reported that phagocytic indices in irradiated rats (600 r) were decreased on the third postirradiation day and returned to normal by the fourth postirradiation day.

C. Effect of x-irradiation on intracellular digestion. The effect of x-irradiation on phagocytic activity, as shown in the preceeding review of literature, is highly controversial, although the majority of investigations

indicate that phagocytic activity is not suppressed by x-irradiation in the sublethal range.

The observation of an increase in the number of organisms in the blood stream of x-irradiated animals 8 hours after the injection of Klebsiella pneumoniae, as reported by Gordon et al., (1955), suggested that the ability of the phagocyte to destroy microorganisms could be impaired following x-irradiation. Fitch et al., (1953) reported that during the 24 hours following the intravenous injection of I-131 labeled typhoid vaccine in rats, 47% of the I-131 activity was excreted by x-irradiated animals as compared to 68% I-131 excretion by the normal animals. This difference in percentage excretion remained marked until the fourth day after injection.

Donaldson, Marcus, and Gyi (1954) reported that x-irradiation of mice (350 r) resulted in decreased intracellular digestion of the nuclei of chicken erythrocytes by peritoneal phagocytes from the sixth through the fifteenth postirradiation days. When the x-irradiation dose was increased to 450 r, this depression of intracellular digestive capacity was prolonged through the twenty-second postirradiation day. Similar studies carried out employing Candida guilliermondii as a living test organism also indicated that phagocyte digestive activity in mice is impaired following exposure to x-irradiation in a dose of 350 r (Donaldson and Marcus, 1956). It is of interest, in connection with these

studies, to note that leucocytic extracts from x-irradiated animals demonstrated no bactericidal activity against Staphylococcus aureus when tested on the third postirradiation day while extracts from control animals or rats 1 day after x-irradiation were actively bactericidal (Fishman and Shechmeister, 1955).

It appears from the limited material available and reviewed in this section that intracellular digestive function of phagocytes is impaired by whole body x-irradiation.

D. Effects of chronic exposure to ionizing radiation on phagocyte function. Although literature concerning the hematologic effects of single exposure to ionizing radiation is overwhelming, similar studies employing multiple exposure is rare. The hematopoietic system of mice and rabbits exposed to periodic doses of gamma radiation, until accumulated doses reached as high as 5,800 r, is comparatively radiation resistant as compared with the hematopoietic system of guinea pigs. It appears then that the dose response relationship is in part dependent on the species of animal employed (Lorenz et al., (1954). Repetitive exposures to 75 r per week of gamma irradiation for different weeks caused a distinct decrease in the number of circulating leucocytes in the rat (Baum et al., 1955). Miller (1956) reported that mice exposed to 60 r per day of gamma irradiation can accumulate 2 or 3 thousand r before their resistance

to experimental infection is much reduced, despite the development of severe leucopenia. Studies concerning the effects of repeated exposure to x-irradiation on both the phagocytic and intracellular digestive (cytopeptic) functions of macrophages will be reported upon in the experimental sections of this thesis.

V. Influence of Immunization on Phagocyte Function

Immunization has long been successfully employed as an effective means of inducing resistance to various types of infectious disease. A few days following an injection of antigen there occurs a so-called "immune response". Currently the characteristic of this response assumed to be most significant is the appearance of specific antibodies in the circulation. Certain findings have indicated that active immunization confers definite alterations in the phagocytes themselves and that these changes in the phagocytic cells are independent of the humoral factors which are of significance in acquired resistance to infectious disease.

Metchnikoff (1905) conceived phagocytes as themselves possessing different degrees of activity in normal and immune states of animals. Wilson and Miles (1956) have stated that, "an immunized animal behaves towards a virulent strain of a particular pathogenic bacterium in the same way as a normal animal behaves towards an avirulent or slightly virulent

strain of the same species". Wright (1923) studied the response of rabbits that had been immunized with a killed culture of pneumococci, to the intravenous injection of virulent pneumococci. Studies of the number of pneumococci per ml of circulating blood at various times after inoculation of a virulent strain revealed that in the immunized animals the organisms completely disappeared from the blood stream within 96 hours after injection whereas in the normal animals the number of organisms in the blood stream first decreased and then reappeared and multiplied until the animals died. Subsequent investigators have made similar observations (Angevine, 1936; Kerby et al., 1950). The enhanced capacity of macrophages to phagocytize microorganisms has been implicated as the cause of such observed differences in normal and immunized animals. Denys and Leclef (1895) after studying the nature of streptococcus immunity in rabbits, indicated that the increased phagocytosis of virulent bacteria following immunization depends upon alterations in the functions of the serum rather than in the phagocytic cells. Furthermore, they presented evidence suggesting that the influence of serum was not one of leucocyte stimulation. Rather, the evidence suggested that the serum acted upon the bacteria rendering them susceptible to phagocytosis. The experiments of Cannon and Pacheios (1930) with staphylococci also demonstrated that specific active immunization promotes phagocytosis by both macrophages and polymorphonuclear leucocytes and that the increase may be attributed to bacteriotropins.

In an attempt to find out which serum component is active in promoting phagocytosis, Mudd et al., (1934) studied various serum fractions obtained from immune animals. The effectiveness of serum components was found to be in the decreasing order of whole serum, euglobin, pseudoglobulin. No phagocytosis occurred after exposure to the albumin fraction.

Street (1942) found that monocytosis occurred in animals during immunization with attenuated pneumococci, and that the monocytes in these animals displayed an enhanced phagocytic capacity which was nonspecific, since the cells ingested even carbon particles and sterptococci more avidly than did those of control animals. Lurie (1939) in his studies of the phagocytic activity of monocytes obtained from animals immunized with BCG or tuberculous animals found that these cells were not only more actively phagocytic for tubercle bacilli but also for carbon particles.

In connection with these findings, it is of interest to recall experiments of Mittermaier (1924). This investigator injected rabbits with staphylococci and 8 days later, when abcesses had been formed, blood was withdrawn and leucocytes were collected and washed. He found that these leucocytes phagocytized the corresponding staphylococci and not streptococci, anthrax or typhoid bacilli. The staphylococci were not phagocytized by washed leucocytes of normal rabbits. Participation of bacteriotropins was ruled out by exposing

normal leucocytes to the serum of the immunized rabbits and then washing them 5 times; such leucocytes did not phagocytize staphylococci. It is also of interest that vaccination of rabbits with killed staphylococci did not enhance their leucocytes' phagocytic capacity. Injection of living staphylococci or of an old broth filtrate was necessary to elicit this phenomenon.

In an attempt to determine the importance of immunization-induced humoral factors on subsequent infection, Rich and McKee (1934) made animals which were immunized against pneumococci, leucopenic by benzol treatment. The aim was to study the effect of immunization independent of leucocytic intervention. They noted that in the immunized leucopenic animals, the proliferating bacteria adhered to themselves and to tissues and were held fixed at the site of inoculation after nonimmune controls had died with septecemia. However the growth of immobilized bacteria proceeded uninterruptedly in the immunized leucopenic rabbits until great colonies and masses of pneumococci had formed at the site of infection. The bacteria eventually penetrated into the blood and lymph streams and the typical animal died with septecemia "even though its plasma is potent in passive protection of non-immune animals possessing leucocytes". The investigators stated that if relatively few leucocytes appeared at the site of infection the bacteria which were opsonized by antibody were rapidly ingested and destroyed and the lesion was sterilized.

This finding suggested that even though there is antibody present, functionally active leucocytes are necessary for clearing the organisms. In this connection it is of interest to note observations concerning development of infections following challenge, in x-irradiated animals previously immunized. Perkins and Marcus (1956) noted that the protective value of preradiation immunization decreased as the radiation dose was increased. Findings that preformed antibody was not affected following x-irradiation exposure (Talmage, 1955) and that x-irradiation suppression of leucocytic functions such as phagocytosis and intracellular digestion was proportional to the radiation dose (Donaldson and Marcus, 1956) implies that the development of infections in immunized x-irradiated animals, which are subsequently challenged with the corresponding organisms, is dependent upon the number of functionally active leucocytes remaining uninjured following x-irradiation exposure. If this speculation is valid, one would then expect that transfusion of functionally active leucocytes following exposure to ionizing radiation might be of value in controlling the infections that develop following radiation injury. In this connection it is of interest to note that Hollingsworth et al., (1956) obtained reduction in the development of bacteremia following radiation injury in rats by transfusion of leucocytes from normal animals.

Following active immunization, phagocytes attain an increased ability to digest the ingested organisms which

are normally resistant to digestion. Lurie (1942) injected lymph node suspensions containing engulfed tubercle bacilli into the anterior chambers of rabbit eyes. He found that the phagocytes from rabbits immunized with BCG vaccine inhibited the multiplication of this organism. Similar observations have been made by Suter (1952) utilizing in vitro technics. This worker demonstrated that monocytes from immunized rabbits or guinea pigs were able to inhibit proliferation of tubercle bacilli. On the other hand, Mackaness (1954) was unable to obtain any difference in the rates of increase of bacterial populations of tubercle bacilli in monocytes obtained from normal and BCG vaccinated animals.

Similar findings have been reported in the literature indicating that certain changes take place in the leucocytes obtained from animals immune to virus infections. Fairbrother (1933) studied the production of immunity to vaccinia virus by mixtures of antibody and virus. By means of intracerebral tests in rabbits he showed a viricidal action of normal, and an even greater action of immune leucocytes on vaccinia virus in the presence of antibody. Similar observations with herpes simplex virus were made by Jamuni and Holden (1934). These investigators found that serum-virus mixtures which were themselves infectious when injected intracerebrally into rabbits, in some experiments were not infectious following mixture with leucocytes. In contrast

to these observations Sabin (1935) found neither evidence of the ability of antibody to exert an opsonic action on vaccinia virus nor evidence that the leucocytes can destroy the virus in the presence of antibody. More recently, Ginder (1955) reported that when leucocytes and macrophages, obtained from fibroma-immune rabbits, were added to antibody-fibroma virus mixtures, a significant increase in the neutralization of fibroma virus was observed with the effect of antibody alone. In addition, he found that immune cell suspensions from peritoneal exudates, regional lymph nodes, buffy coats, spleen and liver were all effective in inhibiting fibroma virus. Studies of the mechanism of action of the immune cells revealed (a) that living cells were essential; (b) that normal cells treated with antibody did not simulate the effects of immune cells; (c) that immune cells contained less preformed neutralizing antibody than an equivalent volume of antiserum; and (d) that inhibition of fibroma lesions was not the result of viral interference.

It is of interest that observations concerning the capacity of macrophages obtained from immunized animals to inhibit infectious disease agents have been made with protozoa as well as with bacteria and viruses. Vischer and Suter (1954) studied the intracellular multiplication of Toxoplasma gondii in macrophages cultivated in vitro. From the observations that macrophages from immune animals combined with antibody were more inhibitory than the antibody alone with normal macrophages,

these investigators suggested the plausibility of the role of cellular factors in infections with Toxoplasma. These results are in agreement with previous findings by Taliaferro (1949) that during the period in which resistance increases macrophages become enormously more active in the destruction of malarial parasites.

It appears therefore that Metchnikoff's concept that macrophages obtained from immune and normal animals possess different degrees of activity is well supported. It further appears that this concept applies, not only to noninfectious particulate matter, but also to certain bacterial, viral or protozoal infections.

VI. Adrenal Cortex and Phagocyte Function

The enhancement of susceptibility to various types of infections by cortisone and ACTH in man and experimental animals has been demonstrated by numerous investigators (Hart and Rees, 1950; Thomas, 1952; Germuth et al., 1952; Robinson and Smith, 1953; Kass and Finland, 1953). This decreased resistance to infections has been suggested to be a result of cortisone induced depression of immunological protective mechanisms of the host. Suppression of inflammatory response (Cummings et al., 1952; Dougherty, 1954; Germuth, 1956) and antibody production (Bjorneboe et al., 1951; Germuth et al., 1952; Fischel, 1953) have been reviewed.

This section reviews the relationship between adrenal cortical hormones and the phagocytic and cytopeptic action of phagocytes.

A. Adrenelectomy. Adrenal insufficiency has been reported to increase susceptibility of animals to a number of infections (Perla and Marmortson, 1941; Robinson et al., 1953; Hill, Brown and Gebhardt, 1954). The mechanisms whereby adrenelectomy induces decreased antibacterial resistance are not known. However, a few investigators have attempted to study the influence of the adrenal cortex on the phagocytic capacity of the reticulo-endothelial system. Gordon and Katsh (1949) reported that splenic uptake of colloidal ThO_2 was less in adrenelectomized rats than in normal animals. Esplin, Marcus and Donaldson (1953) found that adrenelectomy caused a significant reduction in the splenic uptake of ThO_2 in rats but not in mice. Although splenic macrophages uptake was normal in adrenalectomized mice, they found that phagocytosis by peritoneal leucocytes was decreased following adrenelectomy and returned to normal after 6 days. On the other hand Reichard et al., (1956) found a significant increase in the uptake of Au-198 by the spleen and liver of adrenelectomized or hypophysectomized rats. From comparative studies of the deposition of Au-198 and ThO_2 in various reticulo-endothelial organs in adrenelectomized of hypophysectomized rats, these investigators concluded that the pituitary-adrenal axis exerts a regulatory influence on phagocytosis.

However, depending on the type of colloidal particle employed and the particular reticulo-endothelial organ examined, this influence on phagocytosis may be accelerating or inhibiting.

B. Effect of ACTH and cortisone on circulating leucocytes. In order to ascertain the role of circulating leucocytes in resistance to infections following treatment with adrenal cortical hormones, numerous investigators have studied the effect cortisone and ACTH have on phagocytic activity of these cells. Crepea et al., (1951) found that the phagocytic activity of the polymorphonuclear leucocytes obtained from 9 out of 10 patients undergoing cortisone therapy was decreased as compared to normal. Similar depressive effect on phagocytic activity was observed by Rebuck and Mellinger (1953). These findings were supported by the observation of Crabbe (1955a, 1955b), who reported that treatment of rabbits with ACTH and cortisone significantly decreased the phagocytosis of staphylococci by leucocytes obtained from such animals. In another investigation however, he reported that (Crabbe, 1956) cortisone in a daily small dose of .2 mg per kg of body weight, administered subcutaneously in rabbits, enhances the phagocytosis of staphylococci by macrophages. In contrast to the above investigators Mogabgab and Thomas (1952) who induced intracutaneous infection with Group A hemolytic streptococci in rabbits, noted that there are no differences in the phagocytic activity of the leucocytes between the normal and the cortisone treated animals. This observation is supported

by findings of Germuth, (1956) that cortisone did not destroy the ability of the leucocytes to phagocytise viable pneumococci. This investigator favors the view that the lack of phagocytic ability of leucocytes from cortisone treated subjects, as observed by previous investigators (Crepea et al., 1951; Rebuck and Mellinger, 1953), could have resulted from a cortisone induced decrease in numbers of leucocytes rather than a direct effect on leucocytic activity.

C. The effect of ACTH and cortisone on the reticulo-endothelial system. In view of the enhancing effect of cortisone on blood stream infection a number of investigators have studied the effect of cortisone on the reticulo-endothelial system. Clawson and Nerenberg (1953) studied the effect of large doses of cortisone upon the ability of the reticulo-endothelial cells of rats to phagocytize streptococci. These workers reported that the efficiency of the RE cells to engulf bacteria appears to be about equal in cortisone treated and normal rats. However, they noted that the organisms in the treated animals were not completely destroyed, as determined from the number of positive fields, until between 72 and 96 hours after the intravenous injection whereas in normal animals this occurred in between 24 and 48 hours. These workers suggested that the reticulo-endothelial cells in cortisone treated animals are unable to destroy engulfed organisms.

Heller (1955) studied the effect of cortisone on the phagocytic function of the reticulo-endothelial system by determining the disappearance rate of intravenously injected radioactive $CrP\text{-}32_4$ from the blood of rats. He reported that in rats which had received a daily intramuscular injection of 20 mg of cortisone for 3 days, the phagocytic activity of the reticulo-endothelial cells was depressed. Cornwell (1953) reported similar observations using radioactive colloidal gold in rabbits.

On the other hand, Marcus, Esplin and Hill (1953) who studied the 72 hour splenic uptake of colloidal ThO_2 in mice and the peritoneal phagocytosis of Micrococcus aureus in these animals, noted that cortisone in doses of 0.1 mg and 1.0 mg/12 hrs enhanced the phagocytic activity of the macrophages.

Other investigators, whose work is cited below, were unable to demonstrate any alteration in phagocytic activity of reticulo-endothealial cells following cortisone treatment. Benacerraf et al., (1954) reported no effect of cortisone on the granulopectic activity of the reticulo-endothelial cells in mice. This is in agreement with findings by previous investigators who employed bacteria (Martin and Kerby, 1952), colloidal radioactive gold particles (Gell and Hinde, 1953), and india ink (Gamble and Hettig, 1952). Furthermore, phagocytosis of Staphylococcus albus by peritoneal macrophages was not affected by cortisone treatment (Gell and Hinde, 1953). Furthermore, intracellular digestion of chicken erythrocytes

by mouse peritoneal phagocytes has also been reported to be unaltered by treatment with cortisone (Gyi, Donaldson and Marcus, 1955).

It is apparent that the conclusions obtained by different investigators concerning the affects of cortisone and ACTH on phagocyte function are contradictory. These inconsistent results could be due to variation in the hormone doses, the species of animals and the particles utilized to study phagocytosis. With the available evidence it is difficult to state with confidence that phagocytic activities are inhibited by cortisone. Nevertheless the increased susceptibility to infection and the development of bacteremia in cortisone treated animals have been consistently observed. One investigator, (Germuth, 1956) however, stated that "there is no reason to believe that cortisone inhibits phagocytosis in any other way than by suppression of the accumulation of leucocytes during inflammation".

MATERIALS AND METHODS

I. Animals

Adult albino mice (Mus musculus) obtained from a regional source weighing between 20 and 30 gms were employed with the exception of one experiment in which LAf_1 mice weighing 27 to 35 gms were used.* Adult albino rabbits, weighing between 2 and 3 kgs, were purchased from one local source.

II. X-irradiation

A Westinghouse Quadrocondex x-ray machine was used in all experiments. Irradiation factors were; 250 KV, 15 ma, 1.0 mm Al and 0.5 mm Cu filters in addition to an inherent filtration of 2.5 mm Al and 0.25 mm Cu. The distance from focal point to surface on which mice and rabbits were standing was 50 and 105 cm respectively. Mice were irradiated in groups of twelve or less in a cylindrical cardboard container having a diameter of 17 cm and a height of 3 cm. The average dose rate as determined by a Victoreen r meter was 90 r per minute for mice. Measurements were made in air at positions that would be occupied by the center of the mouse's body as it moved about the box during the x-irradiation exposure.

* LAf_1 mice were supplied through the efforts of Lt. Col. Robert Veenstra, Jr. and Dr. Myron S. Silverman of the U. S. Naval Radiobiological Defense Laboratory, San Francisco, California

Rabbits were irradiated, 2 at a time, in a wooden box with dimensions of 8 x 16 x 7.5 in. A wax phantom was placed in the box and the average dose received at the center of the phantom was determined. The dose rate at this point was 26.9 r per minute and each irradiated rabbit received a dose of 600 r.

III. Immunization

The procedure used for immunizing mice consisted of 6 intraperitoneal injections of 0.1 ml of a 5% chicken red blood cell suspension given at 2-day intervals. The chicken erythrocytes were washed in saline 3 times before making up the final suspension. The immunization procedure employed in all rabbit experiments consisted of 6 intraperitoneal injections of 1 ml of a 5% washed chicken erythrocyte suspension given at 3-day intervals.

IV. Radiometric Technic for the Determination of Splenic Uptake of Thorotrast^{R}* in Mice

Mice were sacrificed by ether anaesthesia, spleens removed and their wet weights determined to the nearest milligram. Each spleen was put into a corked test tube in which the spleen was to be homogenized. Before homogenization, 0.5 ml of a 0.3 M

* Thorotrast is a stabilized colloidal solution of ThO_2 (24-26% by vol). The Thorotrast solution employed throughout this experiment was obtained from Tastagar & Co., Inc., Detroit, Michigan.

sucrose solution (isotonic) was added per 100 mg of spleen. Each spleen was then homogenized in a motor-driven tissue grinder. The homogenate (0.2 ml) was pipetted, using either a 0.25 ml Kahn antigen pipette or 0.2 ml pipette, onto an aluminum planchet (Tracerlab). In carrying out these procedures extreme care was taken to prevent loss of fluid by evaporation. The homogenate was plated while rotating the planchet on a turntable exposed to a hot air blower. Under these conditions the preparation dried within 2 to 3 minutes after transfer was completed. The radioactivity of the homogenate was determined using an end window Geiger-Mueller counter (Nuclear-Chicago). The counting equipment was always checked before used with a C-14 standard which gives approximately 5,000 cpm. For each sample, a background count was determined and subtracted from the actual count obtained with the corresponding sample. In all the measurements, duplicate plating of each spleen homogenate was made and the mean taken as the actual activity present.

V. *In Vivo* Technic for the Determination of Intracellular Digestive (Cytopeptic) Activity by Phagocytes

During early investigation on phagocyte function, Metchnikoff injected goose erythrocytes into the peritoneal cavity of guinea pigs and observed the intracellular digestion of these cells by guinea pig phagocytes. A modification of this technic was employed in the present experiments. Adult

albino mice were employed for all in vivo tests. A peritoneal exudate was induced in mice by the injection of 0.5 ml of glycogen-saline (0.01 mg/ml) solution. Thirty-six hours later, at a time when the majority of the cells in the peritoneal exudates were madrophages, 0.5 ml of a 1:20 saline dilution of 4% citrated chicken blood was injected intraperitoneally. At various times after injection of chicken erythrocytes suspension, mice were sacrificed, smears prepared of the peritoneal exudate and stained with Wright's stain. The in vivo data recorded in the tables were collected from the smears prepared 4 hours after the injection of the chicken erythrocytes.

Following the ingestion of the nucleated chicken erythrocytes by the phagocytes, the most marked change was the loss of basophilic staining capacity of the erythrocyte nucleus. The erythrocyte cytoplasm maintained normal staining characteristics after the nucleus had disappeared. Since the nuclei of the noningested chicken erythrocytes in the peritoneal exudate were not morphologically altered, it was assumed that the breakdown of nuclear material of the ingested chicken erythrocyte (Figure 1) was due to intracellular digestion by the phagocytes. The erythrocytes engulfed in 20 to 30 phagocytes were counted by two persons and the percent of cells with abnormal staining characteristics determined.

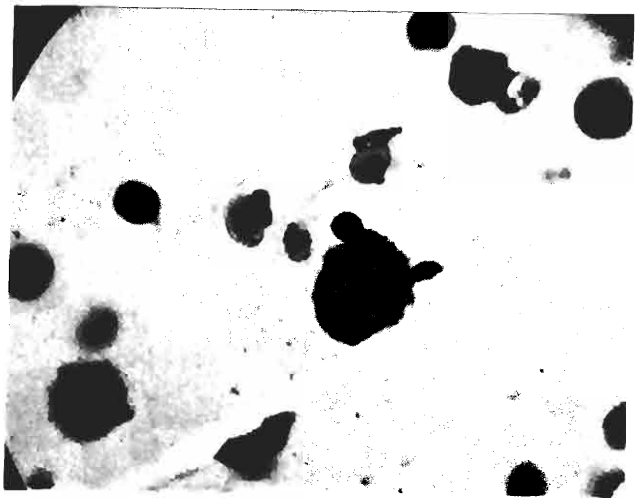


Figure 1. Intracellular digestion of chicken erythrocytes by mouse phagocytes. This slide was prepared from the peritoneal exudate of normal mouse. Chicken erythrocytes in various stages of digestion are seen in the phagocytes.

VI. *In Vitro* Technic for the Determination of Intracellular Digestive (Cytopeptic) Activity by Phagocytes

Phagocytes for the *in vitro* tests were collected from the peritoneal exudates of rabbits 48 hours after the injection of 4 mg of glycogen dissolved in 25 ml of saline. The cells of the peritoneal exudate were washed 3 times with phosphate-buffered saline (pH 7.2) and resuspended in a volume three times the packed cell content. To 0.5 ml of this phagocyte suspension was added an equal volume of heat-inactivated (56°C for 30 min) serum from immunized rabbits. Following the addition of 0.25 ml of a 5% washed erythrocyte suspension to the phagocyte-serum mixture the suspension was incubated at 37°C and smears prepared at various times. The use of antiserum was necessary to obtain consistent phagocytosis *in vitro*.

VII. Other Materials and Methods

The above materials and methods will be considered as standard. Any deviation from the standard will be specified in the discussion of the experimental results. For purposes of continuity, certain other materials and methods employed in the experiments to be reported will be described with the experimental results.

EXPERIMENTAL RESULTS

I. Effect of Single Exposure to X-irradiation on the Phagocytic Activity of the Reticulo-endothelial System

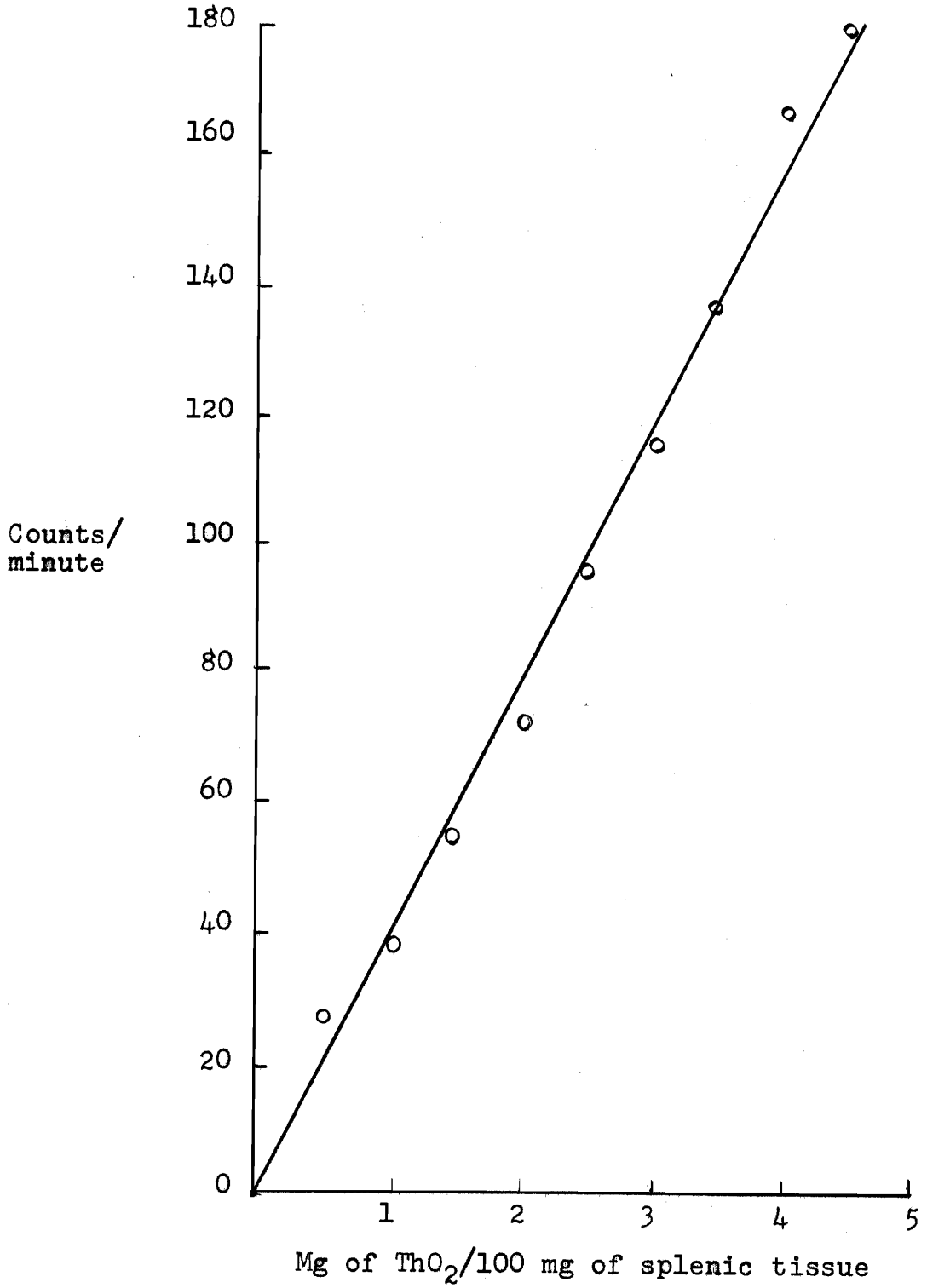
From the material considered in section IV of the literature review of this thesis, it is apparent that the effects of x-irradiation on phagocytosis by circulating and fixed phagocytes are still in doubt. Phagocytosis by reticulo-endothelial cells has been reported to be decreased (Gabrieli, 1953; Taplin et al., 1952), increased (Wish et al., 1951) or unaltered (Barrow et al., 1951, Fitch et al., 1953). In view of these contradictions, studies concerning the effects of x-irradiation on phagocytic activity as measured by the localization in spleen of intravenously injected thorium dioxide were carried out. In these studies different doses of x-irradiation were administered acutely to mice and phagocytic activity determined at different postirradiation periods.

A. Relationship between radioactivity and Thorotrast^{R} concentration. In order to calculate the amount of deposition of Thorotrast^{R} in spleen, it was necessary to know the radioactivity of spleens containing known concentrations of ThO_2. A standard curve was constructed by the following method: spleens were obtained from normal mice and their respective wet weights determined. Various concentrations of ThO_2 (0.5 to 10 mg./ml.) were prepared using 0.3 M sucrose solution as the diluent. The different concentrations of

ThO_2, in 0.5 ml. volumes, were added to give known concentrations per 100 mg. of spleen. The tissues were homogenized, plated on metal planchets (Tracerlab), and the radioactive content of the homogenates determined as previously described. This experiment was repeated three times and the mean values calculated. Figure 2, shows the relation between the radioactivity and the known quantity of ThO_2 present per 100 mg. of splenic tissue. Each point on the graph represents the mean value of three determinations. It is seen that the radioactive content of the homogenates is directly proportional to the amount of ThO_2 within the range of concentration of ThO_2 employed. For this reason correction for self-absorption of the samples was considered unnecessary.

B. Effect of x-irradiation on the localization of ThO_2 in mouse spleens on the second postirradiation day. A group of 30 mice was exposed to a 300 r dose of whole-body x-irradiation. On the second postirradiation day the mice were injected intravenously with 0.2 ml. of ThO_2, diluted 1:2 in saline. At this time a control group and an equal number of nonirradiated mice received the same amount of ThO_2. The mice were randomly divided into three groups, each consisting of 10 x-irradiated and 10 nonirradiated animals. These groups were sacrificed at 2, 6, and 24 hours, respectively, after the ThO_2 injection. These times were chosen because preliminary studies made at 15 minutes, ½ hour, 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours indicated that uptake

Figure 2. Relationship between radioactivity and ThO_2 concentration in splenic tissue.



by spleens of injected ThO_2 follows a linear relationship with time and reaches a maximum at 2 hours. Spleens were removed following sacrifice and each spleen was used as one sample to determine the ThO_2 uptake. Radioactivity of the samples was determined as previously described. This experiment was repeated with mice exposed to 400 and 500 r of x-irradiation. The results of the experiments are recorded in Table 2. Knowing the respective spleen weights, the amount of ThO_2 present was calculated and the values expressed as milligrams of ThO_2 per organ (spleen). The results are shown in Figure 3. It will be noted that the curves describing the data obtained with the test animals are similar in pattern to those of the respective controls. Statistical analyses, using Student's "t" test for unpaired data, show that splenic localization values of x-irradiated animals are the same as those for corresponding control groups.

C. Effect of x-irradiation on the localization of ThO_2 in mouse spleens on the seventh postirradiation day. In the previous experiment it was observed that x-irradiation in doses of either 300, 400 or 500 r had no effect on the splenic uptake in mice of intravenously injected ThO_2 when determined 2 days after exposure to x-irradiation. Since bacteremia and maximum susceptibility to infection in x-irradiated animals develop at the beginning of the second postirradiation week (Miller et al., 1951, Shechmeister, 1954), it was considered desirable to determine the effects of x-irradiation

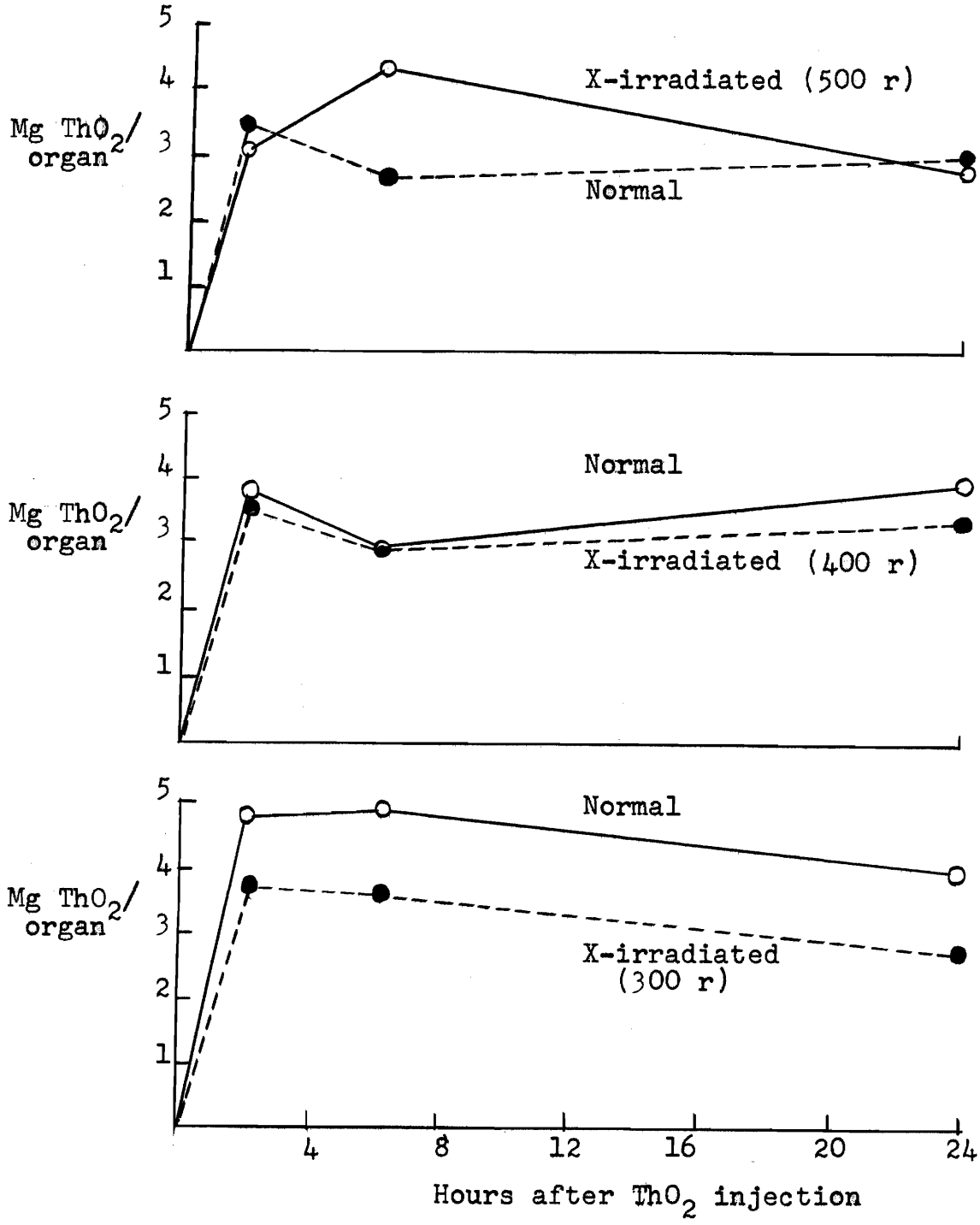
TABLE 2

EFFECT OF VARIOUS DOSES OF X-IRRADIATION ON THE LOCALIZATION IN SPLEEN OF INTRAVENOUSLY INJECTED ThO_2 WHEN TESTED ON THE SECOND POSTIRRADIATION DAY

Expt. no.	X-ray dose	Mg ThO_2 (Mean ± S.E.) per spleen Time after injection of ThO_2		
		2 hours	6 hours	24 hours
I	300 r	3.8 ± .8	3.64 ± .6	2.69 ± .1
	None	4.75 ± .5	4.96 ± .9	3.96 ± .5
II	400 r	3.55 ± .4	2.94 ± .2	3.33 ± .1
	None	3.79 ± .8	2.92 ± .2	3.94 ± .6
III	500 r	3.41 ± .9	2.71 ± .1	2.82 ± .1
	None	3.01 ± .5	4.30 ± .7	2.71 ± .1

Average splenic uptake is calculated from results obtained with 8-10 mice.
Expt. no. - Experiment number.
S.E. - Standard error of the mean.
These abbreviations apply to all the tables that follow.

Figure 3. Effect of various doses of x-irradiation on the localization in spleen of intravenously injected ThO_2 when tested on the second postirradiation day.



on localization of ThO_2 in the spleen at this postirradiation time. An experimental design similar to the one given for the previous experiment was employed except that determination of ThO_2 in spleens was accomplished on the seventh postirradiation day. The results of the experiments are recorded in Table 3. The splenic uptake values are graphically shown in Figure 4. It will be noted that the curves obtained with the x-rayed mice follow patterns similar to those of the respective controls. Statistical analyses using Student's "t" test for unpaired data showed that there is no significant differences in the 2, 6, and 24 hour splenic uptake values between the controls and the 300 or 400 r x-irradiated animals, with the exception of the 2-hour splenic uptake value in the latter group. However, the 500 r dose of x-irradiation was found to depress significantly the splenic uptake of ThO_2 at 2, 6, and 24 hours after injection when compared to the controls.

D. Effects of x-irradiation on the localization of ThO_2 in spleens of LAf_1 mice. In the previous experiment it was demonstrated that a 500 r ($LD_{99}/30$ days) dose of x-irradiation causes a significant depression in the splenic uptake of ThO_2 in the adult albino mice. Since differences in sensitivity to x-irradiation do exist among different strains of the same species the effect of x-irradiation on the localization of ThO_2 in the spleens of LAf_1 mice exposed to a total body dose of 550 r was made. The splenic uptake

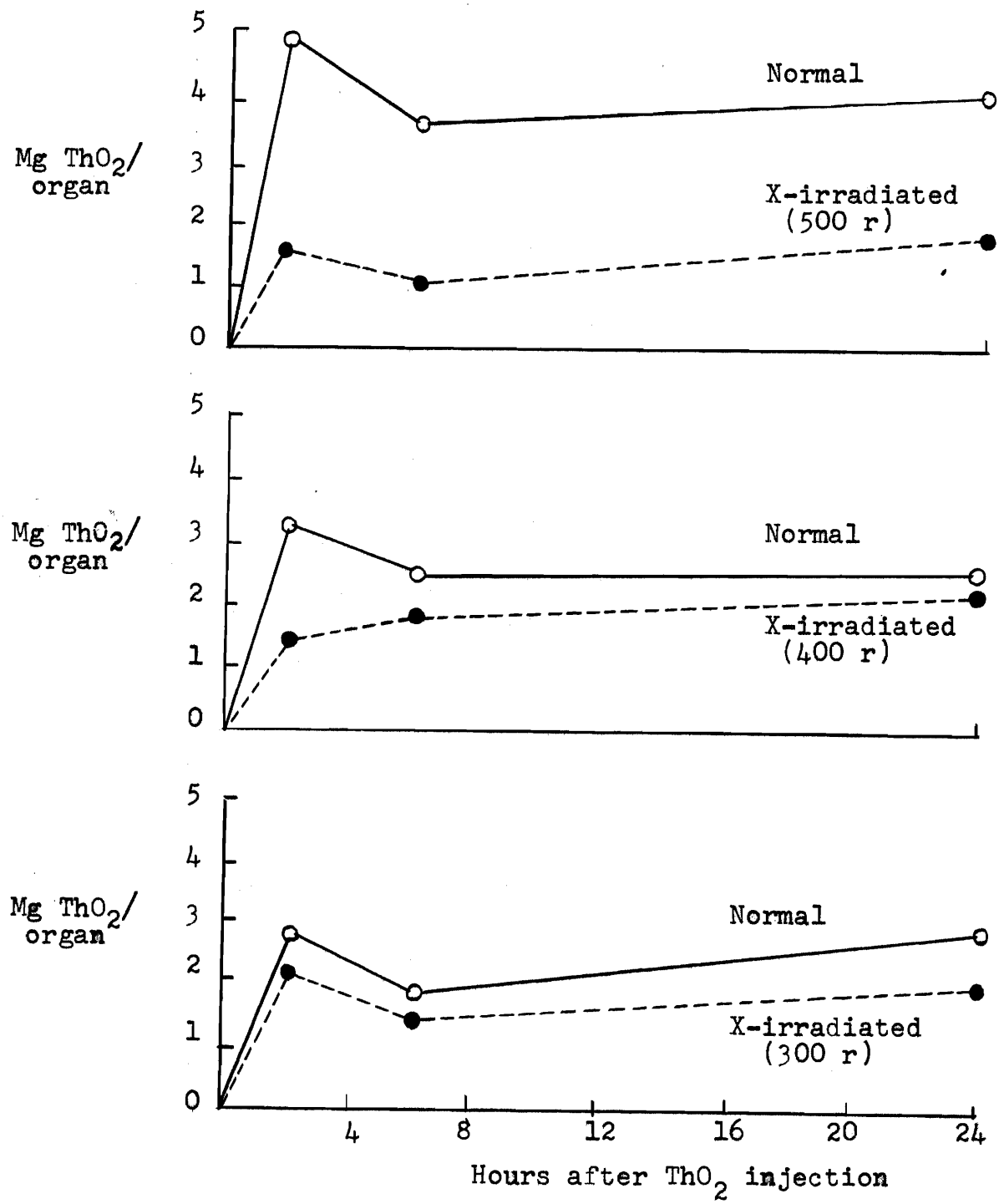
TABLE 3

EFFECT OF VARIOUS DOSES OF X-IRRADIATION ON THE LOCALIZATION IN SPLEEN OF INTRAVENOUSLY INJECTED ThO_2 WHEN TESTED ON THE SEVENTH POSTIRRADIATION DAY

Expt. no.	X-ray dose	Mg ThO_2 (Mean ± S.E.) per spleen time after injection of ThO_2		
		2 hours	6 hours	24 hours
I	300 r	2.19 ± .4	1.56 ± .2	1.98 ± .4
	None	2.83 ± .7	1.93 ± .4	2.76 ± .6
II	400 r	1.42 ± .2*	1.92 ± .1	2.1 ± .5
	None	3.34 ± .6	2.35 ± .4	2.36 ± .1
III.	500 r	1.62 ± .3*	1.14 ± .2*	1.75 ± .7*
	None	4.88 ± .7	3.6 ± .6	3.99 ± .5

*Statistical analysis using Student's "t" test shows significant difference between x-irradiated and control group.

Figure 4. Effect of various doses of x-irradiation on the localization in spleen of intravenously injected ThO_2 when tested on the seventh postirradiation day.



study was carried out seven days after exposure. The results are shown in Table 4 and Figure 5. Statistical analysis showed that a 550 r dose of x-irradiation caused a significant depression of the splenic uptake values at 2, 4 and 24 hours after ThO_2 injection.

II. Effect of Exposure to Chronic Doses of X-irradiation on Phagocytic and Cytopeptic Activity

It has been previously reported (Donaldson, Marcus and Gyi, 1954) that x-irradiation in doses of either 350 or 450 r suppressed the intracellular digestion of chicken erythrocytes by mouse peritoneal phagocytes. In preceding experiments it was demonstrated that x-irradiation in a dose of 500 r caused a significant depression in the uptake, by the mouse spleen, of intravenously injected ThO_2 when tested on the seventh postirradiation day. Although there exists a considerable body of information, (Taliaferro and Taliaferro, 1951, Donaldson and Marcus, 1956) concerning the effects on host defense mechanisms of a single exposure to whole body x-irradiation, relatively little work has been carried out regarding the effects of chronic exposures to ionizing radiation on host defenses. This study was therefore performed to determine the effects of multiple exposures to low doses of x-irradiation on the phagocytic and cytopeptic activities of the phagocytes.

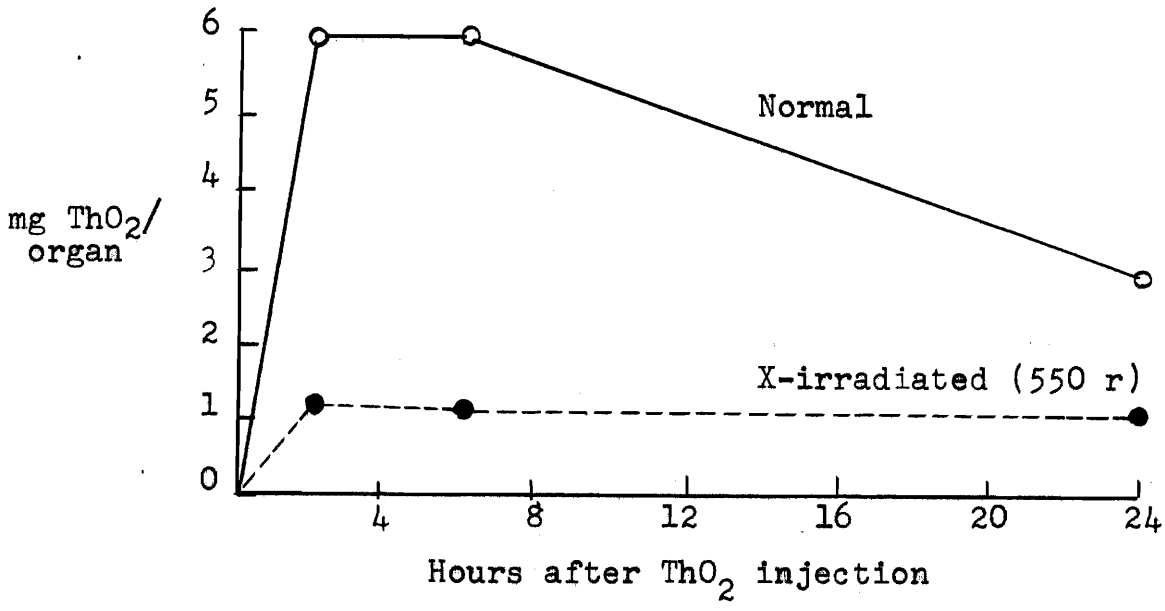
TABLE 4

EFFECT OF A 550 r DOSE OF X-IRRADIATION ON THE LOCALIZATION IN SPLEEN OF INTRAVENOUSLY INJECTED ThO_2 IN LAf_1 MICE WHEN TESTED ON THE SEVENTH POSTIRRADIATION DAY

X-ray dose	Mg ThO_2 (Mean ± S.E.) per spleen Time after injection of ThO_2		
	2 hours	6 hours	24 hours
550 r	1.26 ± .05*	1.12 ± .22*	.92 ± .28*
None	5.90 ± .34	5.94 ± 1.6	2.7± .58

*Statistical analysis using Student's "t" test shows significant difference between x-irradiated and control group.

Figure 5. Effect of a 550 r dose of x-irradiation on the localization in spleen of intravenously injected ThO_2 in LAf_1 mice when tested on the seventh postirradiation day.



A. Effect of chronic doses of x-irradiation on the localization of ThO_2 in spleens. A group of 180 mice were divided into 6 groups. The first group received x-irradiation in a dose of 25 r, 3 times a week for 6 weeks; the second for 8 weeks and the third for 11 weeks. The remaining 3 groups served as controls for each x-irradiated group. The time in days after which measurement of localization of ThO_2 was made and the results obtained are shown in Table 5. Statistical analysis indicated no significant differences in the splenic uptake in x-irradiated animals exposed successively to doses of 25 r, 3 times a week for a period of 6, 8 and 11 weeks; cumulative doses were 450 r, 600 r, and 825 r respectively.

B. Effect of chronic doses of x-irradiation on intracellular digestion by mouse phagocytes. A group of 30 mice were divided into 3 groups. The first and second group received 25 r doses of x-irradiation, 3 times per week for 6 and 8 weeks respectively. The third group served as a control. Seven days after the last radiation exposure, mice were given an intraperitoneal injection of 0.5 ml of citrated chicken blood diluted 1:20 in saline. The animals were sacrificed 4 hours after injection of the chicken red blood cell suspension and smears of the peritoneal exudate were prepared. Intracellular (cytopeptic) digestive activity was determined employing technics already described. The results obtained are recorded in Table 6. Statistical

TABLE 5

EFFECT OF CHRONIC DOSES OF X-IRRADIATION (25 r, 3 TIMES PER WEEK) ON THE LOCALIZATION IN SPLEEN OF INTRAVENOUSLY INJECTED ThO_2

Expt. No.	Time of Exposure (weeks)	Accumulated dose (r)	Day after last x-irradiation dose*	Mg ThO_2 (Mean ± S.E.) per spleen Time after injection of ThO_2		
				2 hours	6 hours	24 hours
I	6	450 r	7	3.35 ± .2	3.5 ± .6	-- --
	none			3.46 ± .2	4.46 ± .1	-- --
II	8	600 r	8	3.9 ± 1.1	3.5 ± .1	4.2 ± .2
	none			2.6 ± 1.2	2.9 ± .6	3.5 ± .8
III	11	825 r	2	5.86 ± 1.2	3.98 ± .6	4.4 ± .9
	none			2.74 ± .2	3.99 ± .3	3.88 ± .2

* Day after last x-irradiation exposure that the localization of ThO_2 was measured.

TABLE 6

EFFECT OF CHRONIC DOSES (25 r, 3 TIMES PER WEEK) OF X-IRRADIATION ON THE INTRACELLULAR DIGESTION OF CHICKEN ERYTHROCYTES BY MOUSE PHAGOCYTES

Time of exposure (weeks)	Accumulated dose, r	Percent digested crbc Mean ± S.E.
6	450	72.5 ± 6.3
8	600	74.2 ± 5.8
None	-	72.0 ± 3.8

crbc - chicken red blood cells.

analysis using Student's "t" test for unpaired data show that there is no significant difference between x-irradiated and control groups in the precent of chicken erythrocytes digested.

III. Effects of Immunization and/or X-irradiation on Cytopeptic Action by Phagocytes

During the course of investigations (Donaldson, Marcus and Gyi, 1954; Donaldson and Marcus, 1956) on the effects of total body x-irradiation on specific mammalian host defenses, it was found that x-irradiation in doses of 350 and 450 r suppressed the intracellular digestion of chicken erythrocytes as well as digestion of viable organisms such as Candida gulliermondii. In view of the findings that phagocyte digestive function was depressed following irradiation injury and since immunization has long been used to enhance host's resistance, studies of the effects of immunization alone or in conjunction with x-irradiation on phagocyte digestive function were carried out with the hope of gaining insight into the relationship between cellular immunity and immunization.

A. Effect of active immunization and/or x-irradiation on the in-vivo intracellular digestion of chicken erythrocytes by mouse phagocytes. Since x-irradiation induces depression of intracellular digestion by peritoneal phagocytes,

an attempt was made to determine whether such function was altered by immunization with chicken red blood cells and further, to determine the effect x-irradiation would have on any immunization-induced alteration. Mice were divided randomly into 6 groups. Three of the groups were immunized with chicken red blood cell suspension following the procedure previously described. Two days after the last immunizing injection one immunized group and one non-immunized group were exposed to a 350 r dose of total body x-irradiation. A second immunized group and second non-immunized group received a 450 r dose at the same time. The remaining immunized and non-immunized groups served as immunized and non-immunized controls respectively. On the sixth post-irradiation day, cytopeptic activity test with chicken red blood cell suspension was carried out employing the technics already described. The results obtained from the peritoneal smears prepared 4 hours after the injection of chicken erythrocyte suspension are recorded in Table 7. The results were subjected to statistical analysis using Student's "t" test. Active immunization significantly enhanced the rate of digestion of chicken erythrocyte by the phagocytes as compared to the normal animals. Furthermore, it was noted that intracellular digestion by phagocytes of immunized-irradiated animals was no different from that seen in nonimmunized irradiated mice. This finding suggests that the immunization-induced increased rate of phagocyte digestion was completely

TABLE 7

EFFECT OF X-IRRADIATION AND/OR IMMUNIZATION ON DIGESTION OF CHICKEN ERYTHROCYTES BY MOUSE PHAGOCYTES

Number of mice	Treatment	Percent digested crbc Mean ± S.E.	Compared to	P* value
9	Control	78 ± 2.2		
12	Immunized	95 ± 1.6	Control	<.001
6	350 r	68 ± 5.0	Control	.1-.05
7	Immunized + 350 r	70 ± 2.0	Control Immunized	<.05 <.001
7	450 r	62 ± 2.8	Control	<.01
8	Immunized + 450 r	65 ± 5.6	Control	<.05 <.001

*P values calculated with Student's "t" test.
Underlined P values are significant at the 5% level.

destroyed by exposure to 350 or 450 r of total body x-irradiation given two days following the last immunizing injection. Microscopic examination indicated that there were more extracellular, that is uningested, chicken erythrocytes in the smears from the nonimmunized animals than in the smears from the peritoneal fluid of the immunized mice. Slides prepared from peritoneal exudates at shorter intervals after the intraperitoneal injection of chicken red blood cell suspension revealed that the free chicken erythrocytes in the exudate from all immunized mice were undergoing lysis. Although x-irradiation decreased digestion in the immunized animals no apparent differences in the extent of uptake of chicken erythrocytes by peritoneal phagocytes from immunized nonirradiated and immunized irradiated animals were seen.

Since specific antibody is known to enhance the phagocytosis of bacteria by macrophages (Neufeld and Rimpau, 1905), it was considered desirable to find out if the increased rate of cytopeptic activity of the phagocytes as observed in this experiment, could be attributed to the presence of specific antibody. Therefore, hemolysin titrations were carried out with the sera of these and other mice that had received similar treatment. Hemolysin titrations were performed following the technic described by Kabat and Mayer (1948). This procedure consisted of making two fold dilutions of heated (56°C for 30 minutes) serum in saline starting with a 1:10 serum dilution. One tenth ml of each serum dilution

was added to a tube that contained 0.1 ml of a 2% suspension of chicken erythrocytes, 0.2 ml of 1:20 dilution of guinea pig complement and 0.4 ml of physiological saline. The mixture was incubated at 37°C for 30 minutes and then stored overnight at 4°C. Hemolysin titers were then recorded. The results obtained are recorded in Table 8. Although no significant depression of antibody titer was noted when x-irradiation followed immunization, there was a slight decrease in titers following 350 r of x-irradiation and this decrease became more pronounced following 450 r. With the exception of one mouse, all the 54 non-immunized (irradiated and non-irradiated) mice showed hemolysin titers of less than 1:10. No correlation was noted between the hemolysin titer and the cytopeptic capacity when the results obtained from individual mice were compared.

B. Influence of x-irradiation and/or immunization on the in-vitro intracellular digestion of chicken erythrocytes by rabbit peritoneal phagocytes. In the preceding experiment it was found that immunization resulted in increased digestion by mouse phagocytes of chicken erythrocytes and that this immunization-induced enhanced cytopeptic capacity of the phagocytes was destroyed by exposure to x-irradiation. The possibility existed that, this increased cytopeptic activity following immunization could be due to the fact that all ingested chicken erythrocytes in the phagocytes from immunized animals were phagocytized at an accelerated

TABLE 8

ANTI-CHICKEN RED BLOOD CELL HEMOLYSIN TITERS OF MICE

Number of mice	Treatment	No. of mice with antibody titers of:					
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320
18	None (Control)	17	1					
17	Immunized			1	5	9	2	
14	Immunized ± 350 r			3	3	7	1	
17	Immunized ± 450 r			5	8	2	2	
18	350 r	18						
18	450 r	18						

rate following contact with antibody. Consequently the average time a chicken red blood cell might have been present in the phagocyte from an immunized animal could be longer than such time in the phagocytic cells of non-immunized animals. In view of these possibilities, in vitro digestion experiments were carried out.

Rabbits were employed in these tests because of the technical difficulty encountered in obtaining a sufficient quantity of peritoneal phagocytes from mice.

In the first experiment the rabbits were grouped as follows: one group was untreated and served as controls, the second group was immunized with chicken erythrocyte, the third was x-irradiated and the fourth was immunized and x-irradiated. The irradiated animals were exposed 7 days after the last immunizing injection. Seven days later chicken erythrocyte digestion tests were carried out employing technics already described. The phagocyte suspension obtained from each rabbit within the group was divided into two aliquots and placed in the serum from an immune and an immune-irradiated rabbit. By utilizing this paired technic it was demonstrated that the digestion of chicken red blood cells was unaltered when the tests were carried out in serum from either an immunized or an immunized-irradiated rabbit (compare figures in column 3 and column 4, Table 9). Comparison of the data recorded in Table 9 reveals that the mean per cent digestion has the same distribution pattern as

TABLE 9

EFFECT OF X-IRRADIATION AND/OR IMMUNIZATION ON IN VITRO DIGESTION OF CHICKEN ERYTHROCYTES BY RABBIT PHAGOCYTES PLACED IN SERUM FROM EITHER AN IMMUNIZED OR IMMUNIZED-IRRADIATED RABBIT

Number of Rabbits	Phagocytes from:	Serum from immunized rabbit Percent digested crbc Mean ± S.E.	Serum from immunized 600 r rabbit Percent digested crbc Mean ± S.E.
5	Control	55 ± 7.3	62 ± 3.7
5	Immunized	68 ± 6.2	66 ± 4.9
5	Immunized+600 r	36 ± 4.1	37 ± 5.8
5	600 r	47 ± 5.0	47 ± 8.8

Underlined mean values are statistically different from "Control" values.

observed in the in vivo tests with mice. With the exception of the results from the immunized-irradiated group, none of the means are statistically different from the control group. This lack of statistical significance could be accounted for in view of the large standard errors for the means.

Other experiments were designed in an effort to minimize these standard errors. The number of rabbits per group was increased and the variable encountered by the use of individual sera was eliminated by pooling the sera from immunized rabbits. Table 10 summarizes the result of these experiments. As in the in vivo experiments it was found that (1) immunization significantly enhanced the rate of digestion of chicken erythrocytes by the phagocytes; (2) x-irradiation alone suppressed digestion by phagocytes and, (3) immunization-induced increase in digestion was completely destroyed by x-irradiation (600 r) which was delivered 7 days following the last immunizing injection.

In order to determine whether the observed increase in digestion which follows immunization is dependent on antibody, the hemolysin titers of these and other rabbits receiving similar treatment were measured. Rabbits were bled by cardiac puncture prior to the collection of peritoneal exudates for digestion tests. Hemolysins determinations were carried out as described in the previous experiment. The results are recorded in Table 11. It was noted that as in the case of experiments with mice, the sera from non-immunized rabbits

TABLE 10

EFFECT OF X-IRRADIATION AND/OR IMMUNIZATION ON DIGESTION OF CHICKEN ERYTHROCYTES BY RABBIT PHAGOCYTES IN VITRO

Number of rabbits	Treatment	Percent digested crbc Mean ± S.E.	Compared to	P* value
8	Control	60 ± 1.3		
9	Immunized	76 ± 2.5	Control	$<.001$
9	600 r	43 ± 2.6	Control	$<.001$
8	Immunized + 600 r	33 ± 4.7	Control Immunized	$<.001$ $<.001$

TABLE 11

ANTI-CHICKEN RED BLOOD CELL
HEMOLYSIN TITRES OF RABBITS

Number of rabbits	Treatment	Rabbits with antibody titres of:							
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640
20	Control	19	1						
19	Immunized					4	3	8	4
16	Immunized + 600 r				2	5	3	3	3
18	600 r	17	1						

and irradiated non-immunized rabbits had titers less than 1:10 with the exception of one from each of the non-immunized and control group which showed a titer of 1:10. The sera of the immunized and immunized-irradiated rabbits (600 r) showed a titer of 1:80 or above except for 2 of the immunized-irradiated (600 r) rabbits which had titers of 1:40.

From the observations that hemolysin titers in the immunized non-irradiated and immunized-irradiated rabbits were no different and that immunized-irradiated animals did not digest chicken erythrocytes any more efficiently than phagocytes obtained from non-immunized x-irradiated animals, it is apparent that the immunization-induced increase in digestion (cytopeptic activity) is independent of antibody action.

IV. The Role of Leucocytes in Resistance to Induced Infections

In previous experiments it was found that active immunization with chicken erythrocytes enhanced the capacity of the phagocytes to digest chicken red blood cells, and that this increased intracellular digestive activity is independent of antibody. As early as 1900 Denys and Kaisin (1893) reported that the pleural exudates of rabbits obtained by injection of dead staphylococci and freed of cells by

centrifugation was bactericidal for living organisms of the same species. Hiss and Zinsser (1908) obtained protective results when extracts of rabbit leucocytes were administered to experimental animals infected with a variety of pathogens. In view of these findings and in an effort to gain more insight into the role of cellular factors in resistance to infection investigations were carried out with leucocytes. Experiments were performed to determine if leucocytes obtained from mice immunized with K. pneumoniae might afford protection to normal animals following challenge with this organism.

A. Resistance of normal mice injected with leucocytes from immunized mice to intraperitoneal challenge with K. pneumoniae. Forty adult mice were immunized with K. pneumoniae vaccine. The immunization procedure consisted of giving 8, 0.1 ml intraperitoneal injections of K. pneumoniae vaccine at 2 day intervals. Vaccines used for immunization were prepared by harvesting 16 to 18 hour tryptose phosphate broth cultures of K. pneumoniae by centrifugation, washing and resuspending in saline. Formalin was then added in a final concentration of 0.5%. Four weeks after the last injection of vaccine each animal was given a 0.5 ml intraperitoneal injection of glycogen saline solution (0.1 mg/ml). Three days later, when the majority of the cells in the exudates were macrophages the mice were sacrificed and the peritoneal cavities opened. Exudates were then collected

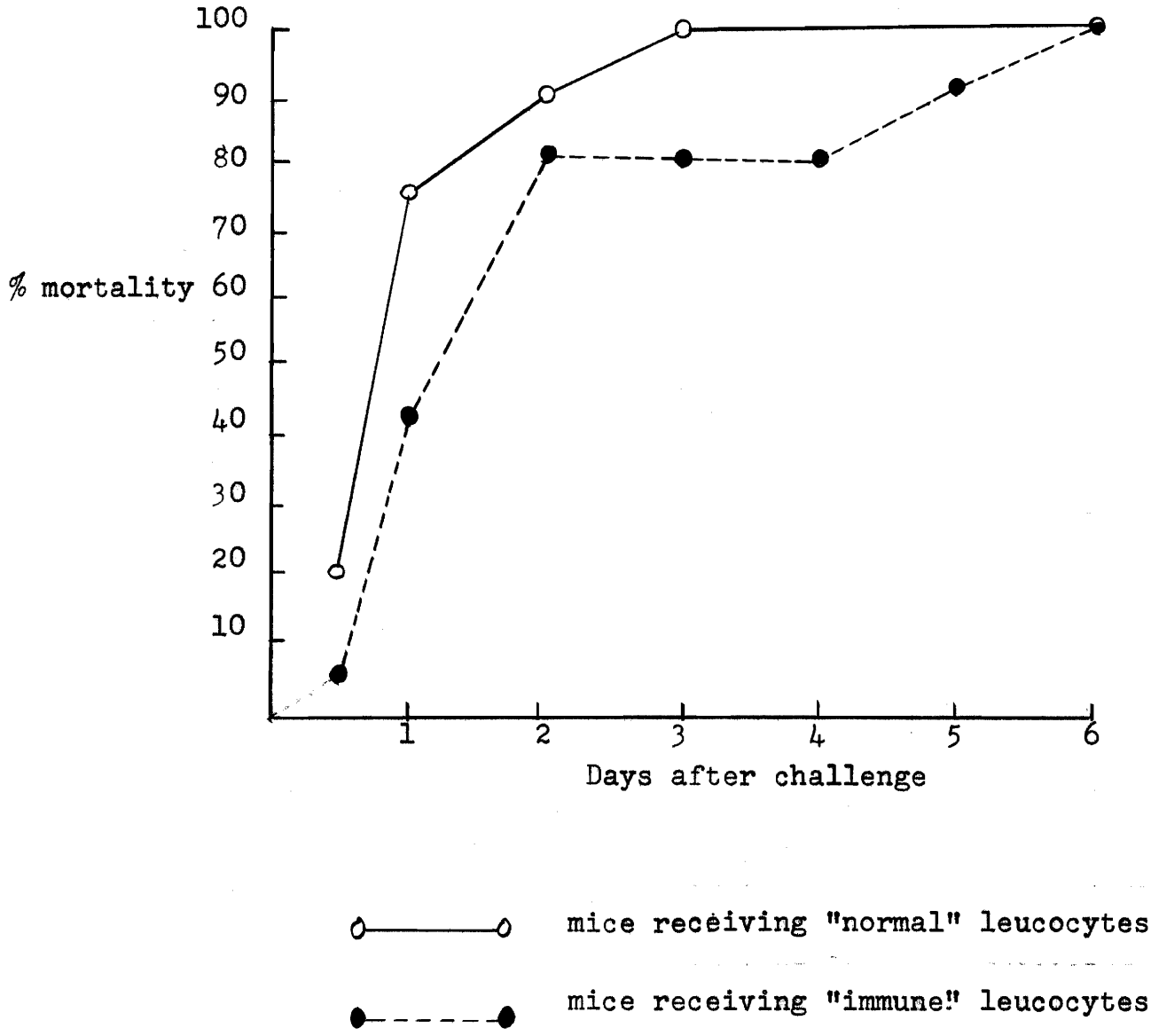
aseptically by washing the peritoneal cavity with phosphate buffered saline (pH 7.2) and resuspended to give a 5% suspension. Using similar procedures leucocytes were also collected from normal animals.

A group of 40 mice was divided at random into two equal groups. Animals in one group each received 0.5 ml intraperitoneally of a phagocyte suspension obtained from immunized animals. The mice in the remaining group each received a similar dose of leucocytes collected from normal animals. Eight hours after the injection of the phagocyte suspension both groups were challenged intraperitoneally with 0.2 ml of a 10^{-4} dilution of an 18 hour broth culture of K. pneumoniae. The mortality results of the experiment are graphically shown in Figure 5. It was found that the mean survival time for normal mice was 1.1 days and for mice which received the intraperitoneal injection of immunized leucocytes 1.8 days. No statistical differences exist in the mortality between the two groups.

V. Effect of Pyrogens on Cytopeptic Activity of Macrophages

Since Metchnikoff's formulation of the phagocytic theory, there has been little effort to search for agents, other than antibody, to enhance phagocytic activity. Within recent years, however, there has been some research in this field. Investigations have been reported in the literature

Figure 6. Resistance of normal mice injected with leucocytes from immunized mice to intraperitoneal challenge with K. pneumoniae.



concerning the ability of certain agents such as surface active substances, ascorbic acid, Piromen^{R}, cortisone, and histamine to either decrease or increase the activities of phagocyte (Nungester and Ames, 1948; Marcus, Esplin and Hill, 1953; Gyi, Donaldson and Marcus, 1955; Kato and Gozsy, 1956). These findings suggest the possibility that the cellular defense mechanism can be stimulated, independent of humoral factors. In the studies mentioned, the ingestive function only was studied. No effort was made to study the effect of the agents employed on digestive function. In view of this, studies of the effect of a pyrogenic agent (Piromen^{R}) on cytopeptic activity of the macrophages were carried out.

A. Effect of Piromen^{R} on intracellular digestion of chicken erythrocytes by mouse phagocytes. In previous studies (Gyi, Donaldson and Marcus, 1955) it was found that Piromen in doses of either 0.1 or 1 ug given subcutaneously, twice daily for 7 days enhanced the capacity of peritoneal phagocytes to digest chicken erythrocytes. However, similar treatment with lower doses (.01 ug) or higher (2 ug) failed to alter the digestive function.

In order to determine the minimum dose of Piromen and days of treatment required for increasing intracellular digestion by phagocytes this experiment was performed varying the days of treatment. Randomly selected mice were divided into groups as shown in Table 12. The first six groups

TABLE 12

EFFECT OF VARYING TREATMENT OF PIROMEN ON INTRACELLULAR DIGESTION OF CHICKEN ERYTHROCYTES BY MOUSE PHAGOCYTES

Dose per Injection (twice daily)	Duration of Treatment (days)	No. of Mice	Percent digested crbc (Mean ± S.E.)	P value
1 ug	3	11	60.2 ± 2.1	< 0.01
1 ug	5	15	64.9 ± 1.6	< 0.001
1 ug	7	12	68.8 ± 1.7	< 0.001
0.1 ug	3	12	55.7 ± 2.8	> 0.1
0.1 ug	5	13	56.9 ± 2.4	< 0.02
0.1 ug	7	15	61.7 ± 2.3	< 0.001
Control (Saline)	-	30	51.2 ± 1.7	—

received Piromen injections subcutaneously in doses of 1 and .1 ug, twice daily for 3, 5, or 7 days respectively. The remaining group served as a saline treated control. Intracellular digestive activity determinations were carried out as previously described. Statistical analyses indicated that Piromen in a dose of 1 ug per injection given twice daily for either 3, 5, or 7 days significantly increased the intracellular digestive activity. However, treatment with similar doses for 3 days did not increase digestion significantly, but a slight trend towards increase in digestion over the control was noted. It is of interest to note the relation between the degree of digestion and the dose and days of treatment. Increasing the dose of Piromen and prolonging treatment brought about a greater increase in the per cent of digestion by phagocytes.

DISCUSSION

In the experiments reported in this thesis it was demonstrated that exposure of mice to x-irradiation in doses of either 300, 400 or 500 r did not affect the phagocytosis, by reticulo-endothelial cells of spleen, of intravenously injected ThO_2 when determined 2 days following x-irradiation. Similar studies carried out on the seventh postirradiation day revealed: (1) that 300 r of whole body x-irradiation had no influence on the 2, 6, and 24 hour splenic uptake values; (2) that 400 r of whole body x-irradiation caused a transient depression of splenic uptake of ThO_2 at 2 hours, but that the 6 and 24 hour values were not different from the controls; and (3) that 500 r of whole body x-irradiation significantly depressed the uptake by the spleen of ThO_2 at 2, 6, and 24 hours after injection when compared to the controls. These findings are similar to those reported by Taplin et al., (1954) who studied the distribution of intravenously injected colloidal prodigiosin in the organs of rabbits. These investigators found that phagocytic function was depressed temporarily at 7 to 10 days after exposure to x-irradiation doses of 800 r or higher, whereas lower doses (300 r) yielded relatively insignificant changes in phagocytic function. Di Luzio (1955) noted depression in the uptake by rat liver of intravenously injected colloidal gold following x-irradiation in

a dose of 1,040 r (LD_{100}) tested 4 days after x-irradiation. A similar observation has been reported by Wilkinson (1954), regarding the effect of x-irradiation (550 r) on the phagocytic activity of the neutrophils obtained from rats. He found that for the first few days following x-irradiation the percentage of phagocytic neutrophils was higher in the blood of x-irradiated animals then in normal animals. However, from the seventh to thirteenth postirradiation day, the ability of the leucocytes from x-irradiated animals to phagocytize was observed to be significantly depressed.

In contrast to these results, Barrow et al., (1951) were unable to demonstrate any differences in the 50% disappearance rates of intravenously injected radioactive gold solution in rabbits exposed to 800 r or higher doses of x-irradiation when determined at different time intervals after x-irradiation for 30 days. On the other hand, Wish et al., (1952) noted that intravenously injected radioactive isotope (I-131) labeled plasma, erythrocytes, and colloidal gold were removed from the circulation more rapidly in the rabbits exposed to an LD_{50} of x-rays than in the normal animals. These results were obtained in the animals during the first postirradiation week.

A few investigators have reported no demonstrable deleterious effect of x-irradiation on phagocytic function. Fitch et al., (1953) injected I-131 labeled typhoid vaccine intravenously into rats 24 hours after exposure to x-irra-

diation (500 r). Radioactivity of organs studied at intervals up to 6 days after injection showed no differences between normal and x-irradiated rats. Esplin et al., (1953) observed no alteration in the splenic uptake of ThO_2 following different doses of x-irradiation in mice (100 to 600 r) tested 3 days after x-ray. These findings are similar to the present observations reported in which x-irradiation in doses as high as 500 r failed to have any injurious effect on phagocytic function when animals were tested 2 days after exposure.

From the data presented in this thesis, it appears that the localization of intravenously injected ThO_2 in x-irradiated mice is a function of both the x-irradiation dose and the postirradiation period of observation. It is apparent therefore that the contradictions in the literature concerning the effects of x-irradiation on phagocytic activity may be due to the differences in the x-irradiation doses employed, the species of animals used and the post-irradiation day that the phagocytic activity was measured. Furthermore it is of interest to note that even among different strains of mice it was found that differences in susceptibility to x-irradiation exist with regard to phagocytosis by the reticulo-endothelial cells. A dose of 550 r (LD_{50}) significantly depressed the phagocytic function of reticulo-endothelial cells of the spleen in LAf_1 mice whereas a comparatively high dose, 500 r (LD_{99}), was required to

suppress the phagocytic function in the adult albino mice utilized in the present investigations. It is significant, in this connection, that Jacobson and his associates (1954) also noted differences in sensitivity to x-irradiation within the same species.

In comparison with other host defense mechanisms such as antibody formation, phagocyte digestion, the properdin system, bactericidal action of serum and leucocytic components, the phagocytic function of reticulo-endothelial cells seems to be comparatively resistant to x-irradiation in the range of minimal to sublethal exposures. The lack of experimental support for any deleterious effect of x-irradiation in the sublethal range on phagocytic activity of reticulo-endothelial cells suggests the importance of other host defenses, such as phagocyte digestion, bactericidal action of leucocytic extracts, the properdin system and the capacity for antibody formation following such x-irradiation exposures. It is of interest, however, to note that depression of phagocytic activity following exposure to lethal doses of x-irradiation occurs during the postirradiation periods when development of bacteremia and susceptibility to infection are known to be at a maximum.

In another experiment (p. 59) it was reported that the intracellular digestion of chicken erythrocytes by peritoneal phagocyte obtained from x-irradiated rabbits was impaired following exposure to x-irradiation. These findings are in

agreement with the previous observations in mice concerning depression of intracellular digestion following x-irradiation (Donaldson, et al., 1955). The observations that irradiation injury resulted in decreased phagocyte digestive capacity and that this suppression of intracellular digestion occurred at times when the x-irradiated animal was more susceptible to infection, suggested the importance of this defense mechanism in the resistance of the irradiated host to infection following exposure to x-irradiation in the sublethal range. It is difficult to evaluate the relative significance of the phagocytic activities in irradiation injury since x-irradiation exerts detrimental effects on other host defenses such as antibody production, phagocyte migration, bactericidal action of serum and leukocytic extracts, properdin system and mechanical fascial barriers. Such x-irradiation induced alterations in host defenses may be the consequence of the action of ionizing irradiation on similar or identical physiological mechanisms, but due to lack of adequate information it is difficult to evaluate the relative importance of any specific postirradiation alteration on the enhanced susceptibility of the irradiated mammalian host to infection.

In assessing effects of repeated exposures to low doses of x-irradiation, certain factors such as degree of injury, regeneration of injured cell, if reparable, and replacement by a new normal cell, should be taken into consideration.

One would expect the dependency of resultant injury to rest on the balance between the magnitude of injury and the rate of repair. From observations of shortening of life span following long-term exposure of mice to x-rays, Blair (1952) concluded that "an animal subjected to an acute lethal dose dies principally of reparable injury, while that subjected to prolonged chronic dosage suffers life shortening due principally to accumulated irreparable injury". Blair further suggested that recovery from injury induced by chronic exposure is not complete and that each successive insult leaves an additional residuum of permanently irreparable tissue, the accumulation of which leads to decreased efficiency of the organism as observed by shortening of life span, genetic effects, and increased incidence of neoplastic diseases. This hypothesis formulated by Blair can be extrapolated to the effect of multiple exposures on host defense mechanisms. In the present investigation mice exposed to x-irradiation in single doses of 25 r at intervals of 2 days, until accumulated doses reached as high as 600 to 825 r, failed to show alterations in the phagocytic activity of reticulo-endothelial cells. In addition to the resistance of the phagocytic system of reticulo-endothelial cells to such exposures, it was also found that intracellular digestion of chicken erythrocytes by peritoneal phagocytes obtained from such animals was also unaffected when studied 7 days following the last x-irradiation exposure. A related observation was reported by Lorenz and Heston (1954) who

were unable to detect any changes in the hematopoietic system of mice exposed to gamma-radiation in a dose of 4.4 r per day up to total doses of approximately 2,000 r. It seems apparent therefore that as long as there exists equilibrium between the amount of injured tissue and the amount of functional tissue, no detectable impairment of a particular function can be observed. Once the amount of damage exceeds the rate of biologic repair, certain impairment of function will become detectable. Assuming this hypothesis, it may be deduced that the resultant injury following successive exposures to ionizing radiation will be a function of the intensity of radiation dose, the interval between the previous and the following dose, and the duration of such treatment.

It was demonstrated in the present investigation, employing both in vivo and in vitro technics, that active immunization with chicken erythrocytes enhances the intracellular digestion of such blood cells by the peritoneal phagocytes obtained from mice and rabbits. From the assumption that the intracellular digestion by phagocytes is mediated by the enzymes of the phagocytes, it is speculated that this immunization-induced increase in digestion is the result of either adaptive changes or increased concentration or activity of the enzymes of the phagocytes. The problem is unanswered concerning the nature of injury sustained by the phagocytes following whole body x-irradiation. Since

intracellular digestion by phagocytes is depressed by 6 days following x-irradiation but not on the second postirradiation day (Donaldson and Marcus, 1956), and since the enhanced effect of immunization is completely reversed by x-irradiation within this same time interval, it appears that the action of x-irradiation on digestive function is not a direct one, but might be a result of injury to mechanisms involved in intracellular enzyme synthesis.

It was observed that the phagocytes from immunized-irradiated animals did not digest chicken erythrocytes any more efficiently than did phagocytes from non-immunized x-irradiated mice. Despite this, the hemolysin concentrations in the immunized-irradiated animals was significantly elevated. It was further observed that there existed no difference in digestive capacity when the phagocyte suspension from individual rabbits was divided into two portions and placed in the serum of either an x-irradiated immunized rabbit or a non-irradiated immunized rabbit. From these observations it is deduced that the observed immunization-induced alteration in digestive activity is independent of antibody action and is a result of changes in the phagocytic cells. Observations similar to those presented in this investigation, that immunization results in the enhanced ability of the phagocytes to destroy or inhibit multiplication of viable organisms and that this is not dependent on humoral factors, have been reported previously. Lurie (1942)

injected suspensions prepared from lymph nodes containing macrophage engulfed tubercle bacilli into the anterior chambers of rabbit eyes. He found that the phagocytes from immunized animals inhibited the multiplication of the organisms in this avascular area. The importance of the cellular elements in combatting this infectious agent following immunization was decisively stressed by Suter (1953) who utilized in vitro technics and demonstrated that the multiplication of tubercle bacilli was inhibited in monocytes from rabbits or guinea pigs vaccinated with BCG. Both preceding investigators observed that the presence of serum from immunized animals did not influence the multiplication of tubercle bacilli in the phagocytes. These observations with tubercle bacilli were later supported by experiments of Raffel (1955) who found inhibition of multiplication of tubercle bacilli in macrophages obtained from BCG vaccinated guinea pigs. A similar inhibitory effect of macrophages obtained from animals immunized against Brucella abortus has also been reported (Pomales-Lebron and Stinebring, 1957). From these reported observations and those presented in this investigation with chicken erythrocytes, it is apparent that evidence is accumulating to suggest strongly that immunization induces significant changes in the phagocytic cells. Dixon (1954) studied the metabolism of I-131 isotope labeled BSA in normal and immunized animals and reported that the latter catabolized

the subsequent injection of antigen faster than the former. Further investigation into the nature of this rapid catabolism of antigen by immunized animals revealed that it occurred at the same rate in actively and passively immunized animals with comparable levels of circulating antibody. In view of these findings this investigator concluded that circulating antibody was all that was necessary for rapid antigen catabolism and that there is no evidence to support the fact that cellular changes which would increase the rate of antigen catabolism occurred as part of the immune response. It is of interest to note in this connection that Talmage et al., (1951) reported that there is no difference in the metabolism of bovine gamma globulin in the normal and x-irradiated rabbit. These conclusions are in contrast to those presented in this investigation in which intracellular digestion by phagocytes of chicken erythrocytes is inhibited following x-irradiation. It could be that digestion by phagocytes of different antigens (soluble and particulate) involve different mechanisms. In relation to this fact it should be recalled that analogous situation exists in the animals treated with cortisone. Hanan and Overman (1953) reported that cortisone treated rabbits suppressed the formation of antibody to bovine serum albumin (soluble) but had little or no effect on the production of hemolysin to sheep cells (particulate).

Assuming the existence of this property, the mechanisms whereby active immunization results in increased digestive capacity by phagocytes is open to speculation. It is however plausible to assume that this enhancement of cytopeptic activity of phagocytes following immunization is due to the formation of adaptive enzymes similar in nature to those observed in bacteria or yeast cells. Further studies concerning the specificity as well as the role of inducing substrate are necessary.

In order to gain insight into the mechanisms involved in the x-irradiation induced depression of phagocyte digestion, certain agents have been examined for their effect on phagocyte digestion. It was found that treatment with histamine in certain doses caused a significant depression in intracellular digestion by peritoneal phagocytes of chicken erythrocytes (Gyi, Donaldson and Marcus, 1955). Since it has been reported that histamine is released following irradiation injury (Ellinger, 1946; Weber and Steggerda, 1949) the possibility exists that histamine may be of importance in the observed x-irradiation induced suppression of cytopeptic function.

In the experiment performed to determine the resistance of normal mice injected intraperitoneally with phagocytes from mice immunized with K. pneumoniae vaccine to an intraperitoneal challenge with homologous organisms it was found that no significant differences existed in the overall

mortalities between the mice that received "immunized" leucocytes and those receiving "normal" leucocytes. There was however, an indication that leucocytes from mice immunized with K. pneumoniae vaccine slightly prolonged the survival time in the normal mice challenged with this organism. The failure to obtain significant results in the present experiment may have been due to the high challenge dose of organisms employed. Another factor which must be considered here is the time of collection of leucocytes after the last immunizing injection. In the experiment performed, these cells were collected one month after the last immunizing injection. If the hypothesis that increase in intracellular digestive capacity by phagocytes following immunization is a result of adaptive enzyme formation, the enhanced activity may be transient in nature and might have decreased to a significant extent during this period of time. In view of these facts further experiments are necessary using suitable challenge doses and employing leucocytes obtained from animals more recently immunized. The various aspects of dose and response remain therefore to be more thoroughly investigated.

It is of interest to note that Piromen in certain doses possesses opposite physiological effects to x-irradiation. Piromen causes leucocytosis (Windle et al., 1950), x-irradiation causes depression of leucocytic response (Cronkite and Bond, 1956). Piromen induces hematopoiesis and splenic

hyperplasia (Berger et al., 1956); x-irradiation causes aplasia of hematopietic organs and splenic parenchymatous destruction (Cronkite and Bond, 1956). In addition to these phenomena, from the present experiments it appears that Piromen in doses that increase intracellular digestion and x-irradiation in doses that decrease intracellular digestion have opposite effects on leucocytosis. Furthermore, it is of interest to note that x-irradiation decreased properdin levels (Pillemer et al., 1954) and that certain lipopolysaccharides obtained from various gram negative organisms, including the lipopolysaccharide obtained from Pseudomonas aeuroginosa causes increase in properdin levels (Landy, 1956). The significance of these observations is open to speculation at the present time.

In view of the opposite effects of Piromen and x-irradiation, a few investigators have tested to see if Piromen affords protection against radiation injury. Donaldson (1954) failed to obtain any significant decrease in mortality in mice treated with Piromen in doses of 2.5, 5 and 10 ug. Mefferd, et al., (1953) reported that intraperitoneal injection of 0.1 ug of Piromen per day per mouse for a 5 day period prior to x-irradiation resulted in a significant increase in the mean survival time of 550 r x-irradiated mice. If administered after x-irradiation Piromen failed to alter survival time. Oral administration of large doses of Piromen resulted in fewer survivals than in the untreated

control mice. It seems apparent that the dose of Piromen employed is important. Further work is necessary to determine whether Piromen in doses that enhance phagocyte intracellular digestion will afford protection against endogenous infections such as those observed in x-irradiation injury.

SUMMARY

1. Total body x-irradiation of mice in doses of either 300, 400 or 500 r does not affect the phagocytosis by reticulo-endothelial cells of spleen of intravenously injected ThO_2 when determined 2 days following x-irradiation. Similar studies carried out on the seventh postirradiation day revealed (1) that 300 r of whole body x-irradiation had no influence on the 2, 6 and 24 hour splenic uptake values; (2) that 400 r of whole body x-irradiation caused a transient depression of splenic uptake of ThO_2 at 2 hours but 6 and 24 hour values were not different from the controls and (3) that 500 r of whole body x-irradiation significantly depressed the uptake by the spleen of ThO_2 at 2, 6 and 24 hours after injection when compared to the controls. These observations indicated that the effect of x-irradiation on phagocytosis by reticulo-endothelial cells is a function of x-irradiation dose and the postirradiation period of observation that phagocyte function was measured.
2. A dose of 550 r (LD_{50}) significantly depressed the phagocytosis of ThO_2 by reticulo-endothelial cells of spleen in adult LAf_1 mice whereas a comparatively high dose, 500 r (LD_{99}) is required to suppress the phagocytic function in adult albino mice.
3. Chronic exposure of mice to x-irradiation in doses of 25 r at intervals of 2 days, until accumulated doses reached as

high as 600 to 825 r, failed to yield alterations in the phagocytic activity of reticulo-endothelial cells or the cytopeptic activity of peritoneal phagocytes. It is possible that the resultant injury following successive exposures to ionizing radiation is a function of the intensity of the radiation dose, the interval between the previous and the following dose, and the duration of such treatment.

4. X-irradiation in doses of 350 or 450 r in mice significantly depressed the intracellular digestion of chicken erythrocytes by peritoneal phagocytes when studied on the sixth post-irradiation day. Similar observations were obtained when rabbits were exposed to 600 r of x-irradiation and cytopeptic activity was determined on the seventh postirradiation day.

5. Immunization with chicken erythrocytes caused an increase in the digestion of these cells by the phagocytes of mice and rabbits. This immunization induced increase in digestion was completely reversed by x-irradiation although x-irradiation did not appreciably alter the hemolysin titers of the immunized animals.

6. <u>In vitro</u> experiments concerning phagocyte digestive activity carried out in the presence of serum from immunized and immunized-irradiated rabbits showed no difference in digestive activity. Furthermore the finding that the phagocytes from immunized-irradiated animals did not digest chicken erythrocytes any more efficiently than phagocytes from non-

immunized x-irradiated mice even though the hemolysin titers were comparable in the immunized and immunized-irradiated animals suggests that the salutary effect of immunization and the detrimental effect of x-irradiation on phagocyte digestion were due to alterations in the phagocytes themselves and not dependent on antibody which appeared following immunization.

7. Piromen, a polysaccharide extracted from Pseudomonas aeuroginosa, in doses of 1 ug per injection given subcutaneously in mice twice daily for 3, 5 or 7 days, significantly increased the cytopeptic activity of peritoneal phagocytes in mice. Similar treatment employing a dose of 0.1 ug also increased the cytopeptic activity when animals were treated for 5 or 7 days but no increase in intracellular digestion was noted when treatment was carried out for only 3 days.

8. Studies made of the resistance of normal mice injected intraperitoneally with phagocytes from mice immunized with K. pneumoniae vaccine to an intraperitoneal challenge with the homologous organism indicated that, although there is no significant difference in their overall mortalities, mice treated with leucocytes from immunized mice had a mean survival time slightly longer than the normal mice.

REFERENCES

Angevine, D. M. 1936 Fate of virulent hemolytic streptococcus injected into skin of normal and immunized rabbits. J. Exper. Med. 64:131.

Aschoff, L. 1924 Lectures on Pathology, Paul B. Hoeber, Inc., New York.

Barrow, J., Tullis, J. L. and Chambers, F. W. 1951 The effect of total body x-radiation, antistine, and pyribenzamine on the phagocytic function of the reticulo-endothelial system in rabbits injected intravenously with radioactive colloidal gold. Am. J. Physiol. 164:823.

Baum, S. J., Kimeldorf, D. J. and Jacobsen, E. M. 1955 Effect of repetitive exposure to gamma rays on the hematopoietic system of the rat. Blood 10:926.

Benacerraf, B., Halpern, B. N., Biozzi, G. and Genos, S. A. 1954 Quantitative study of the granulopectic activity of the reticulo-endothelial system III. The effect of cortisone and nitrogen mustard on the regenerative capacity of the R.E.S. after saturation with carbon. Brit. J. Exper. Path. 35:97.

Bennett, I. L. Jr. and Beeson, P. B. 1954 Bacteremia: A consideration of some experimental and clinical aspects. Yale J. Biol. and Med. 26:241.

Berger, A., Elenbogen, G. D. and Ginger, L. G. 1956 Pyrogens, Advances in Chemistry. Series 16:168.

Berry, J. L. and Spies, T. D. 1949 Phagocytosis. Medicine 28:239.

Bjørneboe, M., Fischel, E. E. and Stoerk, H. C. 1951 The effect of cortisone and adrenocorticotrophic hormone on the concentration of circulating antibody. J. Exper. Med. 93:37.

Bloom, W. 1948 Histopathology of Irradiation from External and Internal Sources. McGraw Hill, New York.

Bordet, J. 1939 Traite de L'immunite dans les Maladies Infectieuses. Masson, Paris.

Bowers, J. Z. 1951 Acute radiation syndrome. J. Am. Med. Assoc. 145:63.

Brecher, G., Endicott, K. M., Gump, H. and Brawner, H. P. 1948 Effects of x-ray on lymphoid and hemopoietic tissues of albino mice. Blood 3:1259.

Bubel, C. and Wilcox, W. 1957 Personal communication.

Bull, C. G. 1915 The fate of typhoid bacilli when injected intravenously into normal rabbits. J. Exper. Med. 22:475.

Burnet, F. M. and Fenner, F. 1949 The Production of Antibodies Macmillan and Co. Ltd., Melbourne.

Cannon, P. R. and Pacheco, G. A. 1930 Studies in tissue immunity; cellular reactions of skin of guinea pig as influences by local active immunization. Am. J. Path. 6:749.

Chrom, S. A. 1935 Studies on effect of roentgen rays upon intestinal epithelium and upon reticulo-endothelial cells of liver and spleen. Acta Radiol. 16:641.

Clawson, B. J. and Nerenberg, S. T. 1953 The effect of large doses of cortisone upon the ability of the reticulo-endothelial cells to phagocytise streptococci. J. Lab. and Clin. Med. 42:746.

Cornwell, S. 1953 Studies of reticulo-endothelial function with colloidal gold. Anat. Rec. 115:295.

Crabbe, J. 1955a The effects of cortisone on the phagocytic activity of rabbit macrophages to staphylococci. Acta Endocrinol. 18:251.

Crabbe, J. 1955b The effects of ACTH on the phagocytic activity or rabbit macrophages to staphylococci. Acta Endocrinol. 18:256.

Crabbe, J. 1956 Enhancing action of small doses of cortisone on macrophage phagocytosis of staphylococci in rabbits. Acta Endocrinol. 21:41.

Crepea, S. B., Magnin, J. E. and Seastone, C. V. 1951 Effect of ACTH and cortisone on phagocytosis. Proc. Soc. Exper. Biol. and Med. 77:704.

Cronkite, E. P. and Bond, V. P. 1956 Effects of radiation on mammals. Ann. Rev. Physiol. 18:483.

Cummings, M. M., Drummond, M. C., Michael, M. Jr. and Bloom, W. L. 1952 The influence of cortisone on artificially induced peritoneal exudates. Bull. John Hopkins Hosp. 90:185.

Denys, J. and Kaisin, A. 1893 La Cellule 9:335. Original not seen. Cited in Zinsser, H., Enders, J. F. and Fothergill, L. D. 1940 Immunity Principles and Application in Medicine and Public Health. The Macmillan Co., New York.

Denys, J. and Le Clef, J. 1895 Sur le mechanisme de l'immunite chez lapin vaccine contre le streptocoque pyogene. La Cellule 11:175.

Di Luzio, N. R. 1955 Effect of x-irradiation and choline on the reticulo-endothelial system of the rat. Am. J. Physiol. 181:595.

Dixon, F. J. 1954 The metabolism of antigen and antibody. J. Allergy 25:487.

Dobson, E. L., Gofman, J. W., Jones, H. B., Kelly, L. S. and Walker, L. A. 1949 II. The controlled selective localization of radioisotopes of yttrium, zirconium and columbium in the bone marrow, liver and spleen. J. Lab. and Clin. Med. 34:305.

Donaldson, D. M. 1954 Aspects of the relationship between irradiation injury and mammalian host defenses. Ph. D. Thesis, Dept. of Bacteriology, University of Utah.

Donaldson, D. M., Marcus, S. and Gyi, K. K. 1954 Influence of whole body irradiation on intracellular digestion by mouse phagocytes. Fed. Proc. 13:490.

Donaldson, D. M. and Marcus, S. 1956 Aspects of the relationship between irradiation injury and mammalian host defense mechanisms. A Review.School of Aviation Medicine, USAF, Report No. J6 - 50.

Dougherty, T. F. 1954 The mechanisms of action of adrenocortical hormones in allergy. Progress in Allergy 4:319.

Downey, H. 1938 Handbook of Hematology. Vol. II. Paul B. Hoeber, Inc. New York.

Dubos, R. J. 1945 The Bacterial Cell. Harvard University Press, Cambridge.

Dubos, R. J. 1953 Effect of Ketone bodies and other metabolites on the survival and multiplication of staphylococci and tubercle bacilli in vitro. J. Exper. Med. 98:145.

Dubos, R. J. 1954 Biochemical Determinats of Microbial Diseases. Harvard University Press, Cambridge.

Ellinger, F. 1946 Protective action of desoxycorticosterone acetate against x-ray induced liver changes. Science 104:502.

Esplin, D. W., Marcus, S. and Donaldson, D. M. 1953 Effects of x-irradiation and adrenalectomy on phagocytic activity. J. Immunol. 70:454.

Fairbrother, R. W. 1933 The production of immunity to vaccinia virus by mixtures of immune serum and virus and the importance of phagocytosis in antivaccinial immunity. J. Path. & Bact. 36:55.

Fenn, W. D. 1922 Theorectical response of living cells to contact with solid bodies. J. Gen. Physiol. 4:373.

Fleming, A. 1922 On a remarkable bacteriolytic element found in tissues and secretions. Proc. Royal Soc. B. 93:306.

Fischel, E. E. 1953 Adrenal hormones and the development of antibody and hypersensitivity. In: The Effect of ACTH and Cortisone on Infection and Resistance, ed. by Shwartzman, G. Columbia University Press, New York, 1953.

Fishman, M., Yunker, R. and Shechmeister, I. 1953 The effects of x-radiation on certain functions of leucocytes. Bact. Proc. p. 52.

Fishman, M. and Shechmeister, I. L. 1955 The effect of ionizing radiation on phagocytosis and bactericidal power of blood. II. The effect of radiation on ingestion and digestion of bacteria. J. Exper. Med. 101:259.

Fitch, F. W., Barker, P., Soules, K. H. and Wissler, R. W. 1953 A study of antigen localization and degradation and the histologic reaction in the spleen of normal, x-irradiated, and spleen-shielded rats. J. Lab. and Clin. Med. 42:598.

Gabrieli, E. R. and Auskaps, A. A. 1953 The effect of whole body x-irradiation on the reticulo-endothelial system as demonstrated by the use of radioactive chromium phosphate. Yale J. Biol. and Med. 26:159.

Gabrieli, E. R. and Cutler, J. L. 1954 Beta irradiation effects on reticulo-endothelial system following intravenous injection of radioactive chromium phosphate. Proc. Soc. Exper. Biol. and Med. 87:661.

Gamble, J. F. and Hettig, R. F. 1952 Effects of cortisone on the removal of india ink by the reticulo-endothelial system. J. Lab. and Clin. Med. 40:801.

Garabedian, G. A. and Syverton, J. T. 1954 Studies on herpes simplex virus. II. An affinity in vitro of herpes virus for rabbit microphages and macrophages. J. Infec. Dis. 96:5.

Gell, P. G. H. and Hinde, I. T. 1953 The effect of cortisone on macrophage activity in mice. Brit. J. Exper. Path. 34:273.

Germuth, F. G. Jr., Ottinger, B. and Oyama, J. 1952 The influence of cortisone on the evolution of acute infection and the development of immunity. Bull. Johns Hopkins Hosp. 91:22.

Germuth, F. G. Jr. 1956 The role of adrenocortical steroids in infection, immunity and hypersensitivity. Pharmacol. Rev. 8:1.

Ginder, D. R. 1955 Resistance to fibroma virus infection. The role of immune leucocytes and immune macrophages. J. Exper. Med. 101:43.

Glenn, J. C. 1946a Studies on the effects of x-rays on phagocytic indices of healthy rabbits. J. Immunol. 52:65.

Glenn, J. C. 1946b Further studies on the influence of x-rays on the phagocytic indices of healthy rabbits. J. Immunol. 53:95.

Gordon, A. S. and Katsh, G. F. 1949 The relation of the adrenal cortex to the structure and phagocytic activity of the macrophagic system. Ann. N.Y. Acad. Sci. 52:1.

Gordon, L. E., Copper, D. B. and Miller, C. P. 1955 Clearance of bacteria from the blood of irradiated rabbits. Proc. Soc. Exper. Biol. and Med. 89:577.

Gyi, K. K., Donaldson, D. M. and Marcus, S. 1955 Influence of cortisone, Piromen, histamine and heparin on intracellular digestion by mouse peritoneal phagocytes. R.E.S. Bull. 1:75.

Grogg, E. and Pearse, A. G. E. 1954 The enzymic and lipid histochemistry of experimental tuberculosis. Brit. J. Exper. Path. 33:567.

Gunn, F. D. and Nungester, W. J. 1936 Pathogenesis and histopathology of experimental pneumonia in rats. Arch. Path. 21:813.

Hollingsworth, J. W., Finch, S. C., and Beeson, P. B. The role of transfused leucocytes in experimental bacteremia of irradiated rats. J. Lab. and Clin. Med. 48:227, 1956.

Hopkins, J. B. and Parker, J. T. 1918 The effect of injections of hemolytic streptococci on susceptible and insusceptible animals. J. Exper. Med. 27:1.

Ingraham, J. S. 1955 Articifial radioactive antigens. IV. The fate of S-35-sulfanil-azo-sheep stromata in x-irradiated, immunized or splenectomized rabbits. J. Infec. Dis. 96:118.

Jaffe, R. H. 1931 The reticulo-endothelial system in immunity, Physiol. Rev. 2:277.

Jamuni, A. and Holden, M. 1934 Role of leucocytes in immunity to herpes. J. Immunol. 26:395.

Jallut, O., Peguidon, L., Lerch, P., Neukomm, S. and Feissly, R. 1955 Speed of disappearance from the blood and distribution in different organs of radioactive colloidal $CrPO_4$ as a function of the injected particle size. R.E.S. Bull. 1:70.

Jochmann, G. in Kolle, W., and von Wasserman, A. 1912 Handbuch der Pathogen Mikroorganismen, Jena, Gustav Fischer, 2nd edition.

Kass, E. H. and Finland, M. 1953 Adrenocortical hormones in infection and immunity. Ann. Rev. Microbiol. 7:361.

Kato, L. and Gozsy, B. 1956 Studies on the effects of phagocytic stimulation on microbial disease VI. Some factors decreasing phagocytic activity of monocytes against tubercle bacilli, strain BCG. Canad. J. Biochem. and Physiol. 34:580.

Kerby, G. P., Holland, B. C. and Martin, S. P. 1950 Quantitative estimation of removal of bacteria from blood by various organs of immunized animal. J. Immunol. 64:123.

Kerby, G. P. and Martin, S. P. 1951 The splanchnic removal of bacteria from the blood stream of leukopenic rabbits. J. Exper. Med. 93:189.

Knott, F. A. and Watt, W. L. 1929 Some effects of deep x-rays upon the cells of the blood. Brit. Med. J. 1:542.

Kohn, H. I. 1951 Effect of x-rays upon hemolysin production in the rat. J. Immunol. 66:525.

Landy, M. 1956 Increase in resistance following administration of bacterial lipopolysaccharides. Ann. N.Y. Acad. Sci. 66:292.

Langhans, T. Beobachtungen über Resorption der Extravasate und Pigmentbildung in denselben. 1870 Arch. Path. Anat. 49:66. (Original not seen. Cited from Mettler, C. C. History of Medicine, P265, The Blakiston Co., New York, 1947).

LeRoy, G. V. 1947 The medical sequelae of the atomic bomb explosion. J. Am. Med. Assoc. 134:1143.

Lorenz, E. and Heston, W. B. 1954 Effects of long-continued total body gram irradiation on mice, guinea pigs, and rabbits. I. Preliminary Experiments. In Zirkle, R. E. (ed.) Biological Effects of External X and Gamma Radiation. Part I. McGraw Hill, New York.

Lurie, M. B. 1939 The mobilization of mononuclear phagocytes in normal and immunized animals and their relative capacities for division and phagocytosis. J. Exper. Med. 69:579.

Lurie, M. B. 1942 The fate of tubercle bacilli ingested by mononuclear phagocytes derived from normal and immunized animals. J. Exper. Med. 75:247.

MacKaness, G. B. 1954 The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. Am. Rev. Tuberculosis 69:495.

Marcus, S. and Donaldson, D. M. 1953 Suppression of normal bactericidal action of rabbit serum following whole body x-irradiation. Proc. Soc. Exper. Biol. and Med. 83:184.

Marcus, S., Esplin, D. W. and Hill, G. A. 1953 Effects of cortisone, asorbic acid and Piromen on phagocytosis in mice. Proc. Soc. Exper. Biol. and Med. 84:565.

Martin, S. P. and Kerby, G. P. 1952 Effect of adrenal hormone overdosage on bacterial removal by the splanchic viscera. Proc. Soc. Exper. Biol. and Med. 81:73.

Mefferd, R. B., Jr., Henkel, K. J. and Loefer, J. B. 1953 Effect of Piromen on survival of irradiated mice. Proc. Soc. Exper. Biol. and Med. 83:54.

Metchnikoff, E. 1905 Resistance to infective Diseases, Cambridge, England, Cambridge University Press.

Miller, C. P. and Hammond, C. W. 1950 The role of infection in radiation injury. Trans. Assoc. Am. Phys. 63:155.

Miller, C. P., Hammond, C. W. and Tompkins, M. 1951 The role of infection in radiation injury. J. Lab. and Clin. Med. 38:331.

Miller, C. P. 1956 The effect of irradiation on natural resistance to infection. Ann. N. Y. Acad. Sci. 66:280.

Mittermaier, R. 1924 Phagozytose and Zellimmunitat. Centralbl. Bakteriol. Orig. XCiii:240.

Manwaring, W. H. and Coe, H. C. 1916 Endothelial opsonins. J. Immunol. 1:401.

Mogabgab, W. J. and Thomas, L. 1952 The effects of cortisone on bacterial infection. J. Lab. and Clin. Med. 39:271.

Mudd, S., McCutcheon, M. and Lucke, B. 1934 Phagocytosis. Physiol. Rev. 14:210.

Myrvik, D. N. and Weiser, R. S. 1955 Studies on antibacterial factors in mammalian tissues and fluids. I. A serum bactericidin for B. subtilis. J. Immunol. 74:9.

Neufeld, F. and Rimpau, W. 1905 Weitere mittheilungen über die Immunität gegen Streptokokken und Pneumokokken. Ztschr. Hyg. u. Infectionskrankh. 51:283.

Nissim, J. A. 1952 Histological changes in the liver in experimental siderosis. J. Physiol. 118:56P.

Nungester, W. J. and Watrous, R. M. 1934 Accumulation of bacteriophage in spleen and liver following its intravenous inoculation. Proc. Soc. Exper. Biol. and Med. 31:901.

Nungester, W. J. and Ames, A. M. 1948 The relationship between ascorbic acid and phagocytic activity. J. Infec. Dis. 83:50.

Nungester, W. J. 1954 Nonspecific factors in immunity. Ann. Rev. Microbiol. 8:363.

Opie, E. L. 1922 Intracellular digestion. The enzymes and antienzymes concerned. Physiol. Rev. 2:552.

Osborne, J. W., Bryan, H. S., Quastler, H. and Rhoades, H. E. 1952 X-irradiation and bacteremia studies on roentgen death in mice. Am. J. Physiol. 170:414.

Panum, P. L. 1874 Das putride Gift, die Bakterien, die putride infection oder Intoxication und die Septicamie. Arch. Path. Anat. 60:301. (Original not seen. Cited from Mettler, C. C. History of Medicine, P. 265, The Blakiston Co., New York, 1947).

Perla, D. and Marmorston, J. 1941 Natural Resistance and Clinical Medicine, Little Brown and Co., Boston, 1941.

Perkins, E. H. and Marcus, S. 1957 Effect of pre-radiation immunization on resistance to aerosol induced infection in x-radiated mice. School of Aviation Medicine, USAF Project Report. (In press).

Pettersson, A. 1905 Ueber die bakteriziden Leukocytenstoffe und ihre Beziehung zur Immutat. Centr. Bakt. 39:423.

Pillemer, L., Blum, L. Lepow, I. H., Ross, O. A., Todd, E. W. and Wardlaw, A. C. 1954 The properdin system and immunity: I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science 120:279.

Pomales-Lebron, A. and Stinebring, W. R. 1957 Intracellular multiplication of Brucella abortus in normal and immune mononuclear phagocytes. Proc. Soc. Exper. Biol. and Med. 94:78.

Ponfick. 1869 Virchows Arch. 48:1. (Original not seen. Cited from Wilson, G.S. and Miles, A. A. Topley and Wilson's Principles of Bacteriology and Immunity (Vol. II, 1955).

Raffel, S. 1955 The mechanisms involved in acquired immunity to tuberculosis. Ciba Foundation Symposium on Experimental Tuberculosis, p. 261.

Raffel, S. 1956 Immunity (properdin agammaglobulinemia, irradiation and immunologic paralysis). Ann. Rev. Med. 7:385.

Rebuck, J. W. and Mellinger, R. C. 1953 Interruption by topical cortisone of leukocytic cycles in acute inflammation in man. Ann. N. Y. Acad. Sci. 56:715.

Reichel, H. A. 1939 Removal of bacteria from the blood stream. Experiments tending to determine the rate of removal of injected bacteria in the blood. Proc. Mayo Clin. 14:138.

Reichard, S. M., Edelmann, A. and Gordon, A. S. 1956 Adrenal and hypophyseal influences upon the uptake of radioactive gold (Au 198) by the reticulo-endothelial system. Endocrinology 59:55.

Rich, A. R. and McKee, C. M. 1934 Study of character and degree of protection afforded by immune state independently of leucocytes. Bull. Johns. Hopkins Hosp. 54:277.

Robertson, O. H., Coggeshall, L. T. and Terrel, E. E. 1933 Experimental pneumococcus lobar pneumonia in dog; pathogenesis. J. Clin. Investigation 12:467.

Robertson, O. H. 1941 Phagocytosis of foreign material in the lung. Physiol. Rev. 21:112.

Robinson, H. J., Mason, R. C. and Smith, A. L. 1953 Beneficial effects of cortisone on survival of rats infected with D. pneumoniae. Proc. Soc. Exper. Biol. and Med. 84:312.

Robinson, H. J. and Smith, A. L. 1953. The effect of adrenal cortical hormones on experimental infection. Ann. N. Y. Acad. Sci. 56:757.

Roser, K. 1881 Beitrage zur Biologie niederster organismen. Marburg. (Original not seen. Cited from Mettler, C. C. History of Medicine, P. 265, The Blakiston Co., New York, 1947).

Rous, P. 1925 The relative reaction within living mammalian tissues. J. Exper. Med. 41:379.

Sabin, A. B. 1935 The mechanisms of immunity to filterable viruses. III. Role of leucocytes in immunity to vaccinia. Brit. J. Exper. Path. 16:158.

Salton, M. R. J. 1953 Cell structure and enzymic lysis of bacteria. J. Gen. Microbiol. 9:512.

Schattenfroli, A. 1897 Uelser die backterienfeind-lichen Eigenschaften der leukocyten. Arch. Hyg. 31:1.

Shechmeister, I. L. and Fishman, M. 1955 The effect of ionizing radiation on phagocytosis and bactericidal power of blood, I. The effect of radiation on migration of leucocytes. J. Exp. Med. 101:259.

Sheppard, C. W., Jordan, G. and Hahn, P. F. 1951 Disappearance of isotopically labeled gold colloids from the circulation of the dog. Am. J. Physiol. 164:345.

Street, J. A. 1942 Studies on the mechanism of species - specific immunity against pneumococcal infection. J. Immunol. 43:53.

Sullivan, F. L., Neckerman, E. F. and Cannon, P. R. 1934 The localization and fate of bacteria in the tissues. J. Immunol. 26:49.

Suter, E. 1953 Multiplication of tubercle bacilli within mononuclear phagocytes in tissue cultures derived from normal and animals vaccinated with BCG. J. Exper. Med. 97:235.

Suter, E. 1956 Interaction between phagocytes and pathogenic microorganisms. Bact. Rev. 20:94.

Taliaferro, W. H. 1949 The cellular basis of immunity. Ann. Rev. Microbiol. 3:199.

Talmage, D. W., Dixon, F. J., Bukantz, S. C. and Dammin, G. J. 1951 Antigen elimination from the blood as an early manifestation of the immune response. J. Immunol. 67:243.

Talmage, D. W. 1955 Effect of ionizing radiation on resistance and infection. Ann. Rev. Microbiol. 9:335.

Taplin, G. V., Finnegan, C., Noyes, P. and Sprague, G. 1954 Blood retention of intravenously injected colloidal prodigiosin in normal and roentgen irradiated rabbits; and index of phagocytic function in the reticuloendothelial system. Am. J. Roentgenol., Radium Therapy and Nuclear Med. 71:294.

Thomas, L. 1952 Infectious diseases (the effects of cortisone and adrenocorticotropic hormone on infection). Ann. Rev. Med. 3:1.

Vischer, W. A. and Suter, E. 1954 Intracellular multiplication of Toxoplasma gondii in adult mammalian macrophages cultivated in vitro. Proc. Soc. Exper. Biol. and Med. 86:413.

Warren, S. L. and Whipple, G. H. 1923 Roentgen ray intoxication. I. Bacterial invasion of the blood stream as influenced by x-ray destruction of the mucosal epithelium of the small intestine. J. Exper. Med. 38:713.

Weber, R. P. and Steggerda, F. R. 1949 Histamine in rat plasma; Correlation with blood pressure changes following x-irradiation. Proc. Soc. Exper. Biol. and Med. 70:261.

Weiss, C. and Czarnetzky, E. J. 1935 Proteolytic enzymes of monocytic and polymorphonuclear pleural exudates. Arch. Path. 20:233.

Wilkinson, M. 1954 Changes in the phagocytic activity of polymorphonuclear leucocytes following total body x-irradiation in the rat. Blood 9:810.

Wilson, G. S. and Miles, A. A. 1955 Topley and Wilson's Principles of Bacteriology and Immunity. Volume II. 4th edition. Williams & Wilkins Co., Baltimore.

Windle, W. F., Chambers, W. W., Ricker, W. A., Ginger, L. G. and Koenig, H. 1950 Reactions of tissues to administration of pyrogenic preparation from a Pseudomonas species. Am. J. Med. Sci. 219:422.

Wish, L., Furth, J., Sheppard, C. W. and Storey, R. H. 1952 Disappearance rate of tagged substances from the circulation of roentgen irradiated animals. Am. J. Roentgenol., Radium Therapy and Nuclear Medicine 67:628.

Wood, W. B. Jr., Smith, M. R., Berry, J. W. and Perry, W. D. 1951 Studies on the cellular immunology of acute bacteremia. Tr. Assoc. Am. Physicians. 64:155.

Wright, H. D. 1923 Experimental pneumococcal septicemia and anti-pneumococcal immunity. J. Path. & Bact. 30:185.

Wright, C. S. and Dodd, M. 1955 Phagocytosis. Ann. N. Y. Acad. Sci. 59:945.

Wyssokowitch, W. 1886 Ueber die Schicksale der in's Blut injicirten Mikroorganismen im Korper der Warmbluter. Ztschr. Hyg. 1:3.

ASPECTS OF THE INGESTIVE AND CYTOPEPTIC ACTION OF PHAGOCYTES

by

Ko Ko Gyi

An abstract of a thesis submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Bacteriology

Approved by the faculty committee in

March 1957

Dr. Stanley Marcus, Chairman, Thesis Committee

University of Utah
1957

Total body x-irradiation of mice in doses of either 300, 400 or 500 r does not affect the phagocytosis by reticulo-endothelial cells of ThO_2 on the second postirradiation day. Similar studies carried out on the seventh postirradiation day revealed that 300 r or 400 r of whole body x-irradiation also had no influence on the phagocytosis whereas a dose of 500 r significantly depressed the phagocytic activity of the reticulo-endothelial cells. A dose of 550 r (LD_{50}) significantly depressed the phagocytosis of ThO_2 in LAf_1 mice whereas a comparatively high dose, 500 r (LD_{99}) is required to suppress the phagocytic function in adult albino mice. These observations indicated that the effect of x-irradiation on phagocytosis by reticulo-endothelial cells is dependent on the x-irradiation dose, the postirradiation period of observation that phagocyte function was measured and the strain or species of animals employed.

Chronic exposure of mice to x-irradiation in doses of 25 r at intervals of 2 days, until accumulated doses reached as high as 600 to 825 r, failed to yield alterations in the phagocytic activity of reticulo-endothelial cells or the cytopeptic activity of the peritoneal phagocytes.

Whole body x-irradiation of mice (350 to 450 r) and rabbits (600 r) significantly depressed the intracellular digestion of chicken erythrocytes by peritoneal phagocytes on the sixth postirradiation day.

Immunization with chicken erythrocytes caused an increase in the digestive activity of phagocytes which was found to be independent of antibody action. The hypothesis was offered that this immunization induced alterations in phagocytes is a result of adaptive enzymes formation.

Piromen in doses of 1 ug per injection given subcutaneously in mice twice daily for 3, 5 or 7 days; or doses of 0.1 ug given for 5 or 7 days significantly increased the cytopeptic activity.

No significant differences in overall mortalities existed between mice treated with leucocytes from immunized mice and those treated with leucocytes from normal mice when challenged with K. pneumoniae.

RESEARCH PROPOSALS

submitted

by

Ko Ko Gyi

in partial fulfilment of
the requirements for the
degree of

Doctor of Philosophy

Department of Bacteriology
University of Utah

March, 1957

Research Proposals

These proposals are put in the form of working hypotheses.

1. The active immunization-induced increase in digestive capacity of phagocytes is a result of adaptive enzyme formation.
2. Experimental animals may be protected against infections (induced endogenously or exogenously) following irradiation by the use of preirradiation immunization followed by postirradiation transfusion of leucocytes obtained from animals immunized against the challenge organism or animals immunized against the organisms that cause bacteremia in the irradiated animal.
3. The effects of x-irradiation on the cytopeptic activity of the peritoneal phagocytes is similar to the effects of such ionizing radiation on fixed reticulo-endothelial cells *in vivo* and *in vitro*.
4. The x-irradiation induced depression of intracellular digestion is a result of the action of histamine or histamine-like substances that is released by disintegration of mast cells following irradiation injury.
5. The temporal correlation of phagocytic and cytopeptic activity and antibody concentration following the injection of particulate and soluble antigen.
6. Corticosteroid hormone-induced decrease in phagocytic activity is a result of the anti-inflammatory affect of hormone; one aspect of this action is a decreased number

of leucocytes migrating into the involved areas.

7. Coagulase positive strains of staphylococci are more readily destroyed following phagocytosis by leucocytes from animals immunized against such coagulase.

8. Certain therapeutic substances which increase phagocytic or cytopeptic action afford protection against endogenous infections that develop following radiation injury or following treatment with high doses of corticosteroid hormone.

9. Increased susceptibility to infection during nutritional deficiencies (avitaminosis, protein deficiency) is a result of either or both decreased phagocytic and cytopeptic capacity of the leucocytes. This decreased capacity is temporally related to increased susceptibility.

10. The salutary effect of spleen shielding or of the administration of splenic extracts to irradiated animals is a function of the ability of such procedures to protect or restore the cytopeptic activity of the leucocytes.