THE PHAGOCYTIC ACTIVITIES OF PERITONEAL MACROPHAGES OF VARIOUS MAMMALIAN SPECIES

A Thesis

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by

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ACKNOWLEDGEMENT

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INTRODUCTION

Following an immunization or an infectious disease man and other animals develop immunity to the associated antigens. After the invasion of the foreign antigens or infectious disease the animal undergoes changes which frequently result in modifications of the function of the reticulo-endothelial system. For the past eighty years immunologists have been concerned with these changes and modifications and have conducted experiments to try and determine the mechanisms that are involved in this acquired state of immunity.

A Russian-born immunologist, Elia Metchnikoff, has been credited with the recognition of the relationship of phagocytosis to natural immunity. In 1882, he observed that starfish larvae possessed a group of highly mobile ameboid cells that congregated at points of inflammation which were induced by inserting thorns into the animals. He observed that these cells engulfed carmine particles and proposed that they would ingest other types of particles as well. His theory was supported by the fact that the outcome of an infectious disease, caused by the yeast Monospora bicuspidata, was directly related to whether or not the phagocytic cells of the water flea, Daphnia, could destroy the yeasts. Metchnikoff quickly expanded his studies to higher animals and found phagocytosis to be an effective natural defense mechanism (Barrett, 1970).

Many different cell types possess a minimal phagocytic ability which can be easily demonstrated in tissue culture. However, this in vitro phagocytic capability of most cells is quite limited, with polymorphonuclear leucocytes and macrophages being, by far, the most phagocytic. The major phagocytic cells involved in the early stages of an inflammation

are polymorphonuclear leucocytes since they are circulated with blood and exhibit a positive chemotaxis towards the inflamed area. Later in the inflammatory process the major phagocytic cell is the macrophage. Macrophages are very important to the living animal in fighting off invading microorganisms.

Studies of the host-parasite relationship in certain bacterial diseases such as tularemia, undulant fever, and tuberculosis which are characterized by the intracellular growth of organisms, has led to the development of the theory of cellular immunity. In broad terms this theory holds that cellular immunity does not depend on the presence of, or formation of, specific humoral antibody but is critically dependent on specific changes in lymphocyte populations.

Much is known in general about phagocytosis and the events that occur during the uptake of particulate matter. However, there is a great deal yet to be learned about the events that occur initially at the cell surface just prior to and during the phagocytic act. Certain chemical substances, isolated and purified, are known to act at the cell surface. These chemical substances appear to combine specifically with receptor sites on the surface of lymphocytes and as a result of this modification, the cell's surface is altered. One of these chemicals, occurring widely in nature, agglutinates cells and has thus been termed a phytoagglutinin or lectin. Concanavalin A, a lectin obtained from the jack bean plant, Concanava ensiformis, was used in this study for exploring events that happen at the cell surface during phagocytosis and the overall changes that occur during the process of phagocytosis.

HISTORICAL BACKGROUND

White cells were first observed in circulating blood around 1770 and approximately one hundred years later their phagocytic ability was first noted. Prior to Metchnikoff's time immunity resulted only from circulating antibody. His observations that thorns and starch grains as well as anthrax bacilli were engulfed by phagocytes of various species soon led to a controversy between supporters of his theory and those people who believed that humoral factors alone accounted for immunity.

The process of phagocytosis, as various investigators have described it, involves the engulfment of particulate matter by active pseudopodia or by invagination of the cell membrane. This is followed by a fusion of the cell membrane around the engulfed particle. A chemotactic response directed toward particulate materials such as bacteria or starch grains is exhibited by polymorphonuclear leucocytes and macrophages (Harris, 1954; Jacoby, 1965). However, in vitro the tissue degradation products are not chemotactic.

Rabinovitch (1967) demonstrated two distinct phases of phagocytosis by using mouse peritoneal macrophages and aldehyde-fixed horse erythrocytes. The first phase, attachment, did not require the addition of serum or divalent cations. The second phase of phagocytosis, ingestion, required the presence of calcium ions but not magnesium ions and was also found to be temperature-dependent. At temperatures below an optimum there was a corresponding drop in phagocytosis (Metzger and Casarett, 1967; Rabinovitch, 1967). Norman (1973) has shown that a competitive inhibition existed between particles with both similar and dissimilar surface characteristics and suggested that these particles competed for a common locus on the

surface of the phagocytic cell.

In metabolic studies of phagocytosis Karnovsky (1962) showed that the oxygen uptake was three times as great in actively phagocytizing macrophages as opposed to resting macrophages. The phagocytic activity of macrophages can be inhibited by exposing macrophages to the glycolytic inhibitors iodoacetate and flouride (Oren, 1963). Oren demonstrated that peritoneal macrophages depend mainly on anaerobic oxidative metabolism by using the inhibitors of oxidative metabolism such as dinitrophenol or cyanide. On the other hand alveolar macrophages derived their energy from oxidative phosphorylation. North (1966) found the enzyme adenosine triphosphatase in the cytoplasmic membranes of guinea pig macrophages and suggested that the membrane-bound ATPase acted as a source of energy for phagocytosis. North (1968) found that ATP was a limiting factor in both spreading and phagocytosis and suggested that opsonins enhance phagocytosis by initiating cell-surface enzymatic reactions which then induced the synthesis of more ATP.

The attachment of glutaraldehyde-treated erythrocytes (GRCs) to mouse peritoneal macrophages in a serum-free medium was decreased if the macrophages were trypsinized (Rabinovitch, 1968). Specific anti-erythrocyte serum aided the attachment of GRCs to either the trypsinized or non-trypsinized macrophages. Thus, it was suggested that different receptor sites may exist on peritoneal macrophages, some effective for GRC and others for antibody coated erythrocytes. In 1967, Perkins reported that different strains of mice had different receptors on macrophages. Macrophages bear a variety of receptors for different classes of antibody. Thus the class and the amount of antibody present may strongly influence the rate and

extent of phagocytosis. Phagocytosis of foreign particles by rabbit peritoneal macrophages depended upon specific natural opsonic antibodies (cytophilic antibody) present in rabbit serum and this accounted for the discriminative phagocytosis by these cells. Thus, opsonins either in the serum or cell bound were essential for phagocytosis (Pearsall, 1970).

Variation of intracellular digestion of particulates was great and depended on the type of organism, dose, degree of virulence, serum factors and many other conditions. Macrophages can digest typhoid organisms intracellularly within six hours while virulent tubercle bacilli, if prevented from multiplying intracellularly, remain viable for days and digestion - when it occurred - was still incomplete after eleven days. Virulent or avirulent avian tubercle bacilli survived for at least twenty-one days after being phagocytized (Jacoby, 1965). One factor for the difference in intracellular digestion may lie in the enzymes of the phagolysosomes. Myrvik (1961) found only small amount of lysozyme content in rabbit peritoneal macrophages and a large amount of lysozyme in rabbit alveolar macrophages. In 1962, in short term cultures, Pavillard and Rowley (in Jacoby, 1965) showed pronounced intracellular bactericidal power of mouse peritoneal macrophages but very little in guinea pig alveolar macrophages even though both were phagocytic. The acid phosphatase activity was higher in the guinea pig alveolar macrophages than in the mouse macrophages. Thus, heterogeneity of macrophages occurred at various levels, among classes of animals and probably species, and also regionally within species.

Recently, the influence of specific chemical configurations at the macrophage surface have been studied by treating these chemical sites in

ways which will either mask or expose them. Certain proteins or glycoproteins capable of combining with these receptor sites on macrophages are known as lectins. These proteins and glycoproteins are found in the seeds of plants, particularly legumes, and possess the ability to agglutinate erythrocytes and other types of cells and had been called, collectively, phytohemagglutinins. Since these agglutinins have also been found in invertebrates and in the lower vertebrates they are now collectively called lectins.

Lectins exhibit many unusual chemical and biological properties. Some lectins are specific in their reactions with human blood groups (ABO, MN, and certain subgroups) and thus have been used in blood typing and in investigating the chemistry of blood group specificity. Certain lectins are mitogenic, i.e. they stimulate the transformation of lymphocytes from "resting" cells into large blast-like cells which may undergo mitotic division. Concanavalin A has also been used as an effective immunosuppressive agent at low dosages and in short term experiments was well tolerated (Markowitz et al., 1968). Lectins have provided a new tool for investigating the structure of the cell surface as they bind specifically to saccharides on the cell surface and their action can be inhibited by simple sugars.

Stillmark, in 1888, was the first to study the action of lectins as he described the phenomenon of hemagglutination by plant extracts. He studied the toxicity of castor beans and found that they contained a highly toxic protein which he called ricin and which would agglutinate human and animal red blood cells (Sharon, 1972).

Concanavalin A obtained from the jack bean, Concavalia ensiformis,

was the first lectin to be crystallized. The crystallized form was obtained by Sumner in 1919, but it was not until 1936 that Sumner and Howell identified the hemagglutinin of jack bean with Con A (Sumner and Howell, 1936).

Con A was not specific for human blood groups but was highly specific with respect to the binding of saccharides; its sugar specificity being for α-D-mannopyranosides. Most of the purified lectins contain covalently bound sugars and are classified as glycoproteins. However, Con A was not a glycoprotein as it did not contain any sugar residues.

Con A in solution consisted largely of dimers at pH values less than six and of tetramers above pH 7. Con A has a molecular weight of 25,500 and as a protomer exists in two forms, one containing an intact subunit and one with a subunit made up of two fragments. The Con A subunit is a globular protein with the predominant structural element being an extended polypeptide chain arranged in two anti-parallel pleated sheets or β-structures (Edelman, 1972). Interactions among the monomers to form dimers and tetramers was contributed almost exclusively by one of the pleated sheets. At least twenty angstroms separates the two metal-binding sites from the saccharide-binding site. Edelman (1972) observed that metal-binding on the saccharide-binding ability of Con A must arise through a structural change in the saccharide-binding site induced by bound metal and that there was also a nonpolar region near the carbohydrate-binding site. Con A was thought to bind di- and oligosaccharides through their nonreducing terminal residues (Edelman, 1972).

The carbohydrate-binding site of the Con A molecule appeared to be directed primarily toward unmodified hydroxyl groups at the C-3, C-4, and

C-6 positions of the six-membered α-D-mannopyranoside or α-D-glucopyranoside ring. The inhibitory action of the saccharide was completely abolished if any modification at these positions were made. It was shown that the oxygen atom of the C-2 position of D-mannose served as a polar binding site for the interaction of the saccharide with the protein. D-mannose, D-glucose, and their derivatives bind to Con A in the C-1 chair conformation and the protein possesses a specific size capable of interaction with the anomeric oxygen atom of the α-linked glycosides of these sugars (Sharon, 1972).

Con A has been shown to agglutinate leukemic cells, cells transformed by polyoma virus, simian virus 40, myxoviruses, chemical carcinogens and X-irradiation. Con A did not agglutinate normal cells or untransformed 3T3 cells under the same conditions. The reversal of agglutination by competition with α-methyl-D-glucopyranoside and the inactivation of agglutination by demetallization indicated that agglutination was produced by Con A by the sites on the protein that were involved in carbohydrate binding. Inbar and Sachs (1969) through competition experiments showed that the surface of transformed cells contained different sites for interaction with Con A and wheat germ agglutinin. After trypsin treatment a decrease in the agglutination of transformed cells was found. Thus treatment with trypsin can remove or destroy sites for Con A on the cell surface. It was assumed that untransformed cells contain the same or similar sites as transformed cells in a cryptic form and that after trypsin treatment these sites were exposed. Different degrees of agglutination were found with the different transformed cell lines tested indicating that different lines of transformed cells may vary in the number of exposed sites (Inbar and Sachs, 1969a). Inbar and Sachs (1969b) suggested that normal cells contained sites in a cryptic form and that in transformed cells these

binding sites were exposed. They measured the number of sites directly by measuring the adsorption of Con A molecules whose metal sites requiring α-methyl-D-glucopyranoside binding were labelled with ^{63}Ni. It was found that eighty-five percent of binding sites for the protein Con A that were exposed on the surface membrane of transformed cells were in a cryptic form on normal cells. This change in structure of the surface membrane resulted in the exposure of surface sites and may have produced the change in cellular regulatory mechanisms that was associated with transformation (Inbar and Sachs, 1969b).

After infection of untransformed cells with SV 40, and normal cells with polyoma virus, a change in the structure from normal to transformed cells occurred several days after the initial viral infection. The several days that were required for the detection of agglutination, and the increase in cell number suggested that the change in structure required cell replication (Inbar and Sachs, 1969a).

Receptor sites present on the surface of transformed cells for different lectins are both chemically and topographically distinct since concentrations of the receptor glycoprotein, which fully inhibited the agglutination of cells by wheat germ agglutinin had no effect on the agglutination of Con A or soybean agglutinin (Sharon, 1972).

Cells which have undergone proteolysis, and normal and transformed cells bind approximately the same amount of Con A. There was no direct correlation between the amount of lectin bound and the agglutinability of the cells. Since both normal and transformed cells bind equal amounts of Con A there was an indication that the increased susceptibility of transformed and trypsinized cells to agglutination was not caused by a

simple unmasking of cryptic receptor sites. Baby hamster kidney cells infected with NDV were susceptible to agglutination five hours after infection and paralleled measurements of cell coat thickness revealed a close temporal relationship between changes in agglutination behavior and reduction in cell coat thickness. Poste and Reeve (1972) found that the agglutination of cells by Con A was not a specific property of cells transformed by oncogenic viruses.

It has also been suggested that agglutination by lectins depended upon the relative distribution of sugar receptors on the cells surface. In normal cells the cells are dispersed, whereas after malignant transformation or proteolysis a redistribution of sites has occurred which has resulted in a clustering of the sites, which was an arrangement very favorable to cell agglutination (Sharon, 1972). The medium in which the cells were grown also effected the binding of Con A (Ben-Bassat, 1971).

Rous sarcoma virus (Schmidt-Ruppin strain) - transformed chick embryo cells were agglutinated by Con A. However, normal cells were also agglutinated. Kapeller and Doljanski (1972) found that wheat germ agglutinin agglutinated normal cells only slightly while it agglutinated transformed cells strongly, and the difference in his system was much more pronounced with WGA than Con A. The toxic effect was also investigated and concentrations of 50-700 μg destroyed thirty-five to fifty-five percent of the cells within the first two hours. Different mechanisms thus characterized the agglutination and toxic reaction since the toxic effect was identical in normal and transformed cells. Changes in the cell membrane caused by the binding of the two sites of Con A to one cell may have resulted in cell death. Why there was no dose effect and why only half of the cells were

effected was not determined. Burger (in Kapeller and Doljanski, 1972) found that surface sialic acid was needed for agglutination, though by itself it was not the receptor site, in cells transformed by both DNA and RNA viruses. However, Moore and Temin (1971) did not find any difference in the agglutinability between normal cells and cells infected or transformed by RNA oncogenic viruses.

Maturation of myxovirus was accompanied by alteration of the cell membrane. Completion of infectious virus particles by the host cell was not a prerequisite to expose a maximal amount of Con A receptors. A strain-dependent difference existed as to what degree receptor sites became exposed at the cell surface, since smaller amounts of Con A were needed with different strains of NDV than others. It was also found that not all lipid-containing viruses were equally capable of rendering their host cells agglutinable by Con A. Although different mechanisms underly the conversions of the cell membrane, sensitivity of the myxovirus-invected cells as well as transformed cells to the same lectin exemplified common surface characteristics (Rott and Klenk, 1972).

The mechanism and mode of action of Con A is still uncertain. Thus, it seemed important to study further the action of Con A on macrophage surface receptor sites and how this lectin might modify the process of phagocytosis by either enhancing or depressing this process.

MATERIALS AND METHODS

Experimental Animals

Rats used in this study were inbred albino animals originally obtained from the Lemberger Company, Oshkosh, Wisconsin. The parental stock of white mice used were obtained from Kansas State University, Manhattan, Kansas. Hamsters were from the Golden Syrian Hamster line and were obtained from the VLK Animal Farm, Marion, Kansas. Young New Zealand white rabbits three months of age were obtained from breeders in Emporia and the surrounding area. The rabbits were sacrificed at 4-8 months of age. Healthy young adults were used in all experimentations.

Sera

Bovine serum obtained locally was processed according to standard procedures. The serum was sterilized by passage through a Millipore filter (HA 0.45), aliquoted in 100 ml quantities, heated at 56^{o}C for 30 minutes to destroy complement, and frozen until needed.

Tissue Culture Medium

Rabbit macrophages were harvested in Hanks' Balanced Salts Solution (BSS) plus heparin (2 USP units per ml) and combiotics (penicillin, 200 units per ml and streptomycin, 100 units per ml), and adjusted to the proper pH (approximately pH 7.0) with 1.4% sodium bicarbonate. After cell attachment to glass the Hanks' BSS was replaced with Eagles Minimum Essential Medium (MEM) containing L-glutamine, combiotics and 20% heat inactivated bovine serum.

Glassware

Glassware was washed in Haemo-sol cleaning solution (Scientific Products, Evanston, Ill.), rinsed in tap water four times, distilled deionized water 4-5 times, and allowed to air dry.

Pipettes were soaked in strong Clorox solution for approximately 24 hours, placed in a pipette washer with Alcotab detergent for 12 hours, rinsed individually in distilled deionized water and allowed to air dry.

Flying coverslips were washed in a dilute solution of Haemo-sol with gently constant agitation and allowed to rinse in tap water for 24 hours. They were then individually rinsed 10 times in 6 consecutive distilled deionized water baths and two - 95% ethanol baths. Coverslips were stored in 95% ethanol until autoclaved and used.

Harvesting of Rabbit Peritoneal Macrophages

Rabbits were injected with 50 ml of sterile mineral oil five days prior to harvest and sacrificed by injecting 10-20 ml of air into an ear vein. The abdominal hair was saturated with Purasan and a single edge razor blade was used to shave the abdominal hair. The outer muscle layer was exposed and 100 ml of Hanks' BSS plus heparin was injected into the peritoneal cavity followed by a vigorous massage of the abdomen to release cells from the peritoneal wall. The remaining muscle layer was opened and the exudate drawn out of the peritoneal cavity with a sterile 50 ml volumetric pipette and placed in a separatory funnel. The separatory funnel was held at refrigerator temperatures ($4^{o}C$) for 10-15 minutes to allow the cells to separate from the oil. The bottom layer, containing cells, was decanted into sterile centrifuge tubes and centrifuged at 1500

rpm in a fixed angle head for five minutes. The supernatant was discarded, and the cells resuspended in Hanks' BSS and centrifuged at 1500 rpm for five minutes. Again the supernatant was discarded, the cells resuspended in 50 ml of Hanks' BSS and a cell count made with a Neubauer hemacytometer. The cells were diluted with Hanks' BSS to a final cell concentration of 1×10^6 cells per ml, planted in flying coverslip tubes and incubated at $37^{\circ}C$. After cell attachment to glass the Hanks' BSS was replaced with Eagles MEM plus 20% heat inactivated bovine serum as the culture medium.

Mice were sacrificed by cervical dislocation while hamsters and rats were killed with ether. The experimental animals were secured to a 12" X 12" board, the skin removed aseptically from the ventral side exposing the abdominal muscles. Three to seven ml of harvesting media (Hanks' BSS), depending upon the species, was injected into the peritoneal cavity and the abdomen massaged to suspend the macrophages. An incision was made in the peritoneal wall and the cell exudate removed by means of a sterile capillary pipette. Exudates from like species were pooled, counted on a Neubauer hemacytometer, diluted with Hanks' BSS to a final cell concentration of 1×10^6 cells per ml. One ml aliquots were planted in flying coverslip tubes and incubated at $37^{\circ}C$.

Phagocytosis of Carbon Particles

Peritoneal macrophages were harvested from mice, hamsters, rats, and rabbits, counted and planted at a concentration of 1×10^6 cells per ml in Hanks' BSS. After cell attachment to glass (1.5 hours) the Hanks' was replaced by Eagles MEM plus 20% serum. At appropriate time intervals the Eagles was removed and 1 ml of Higgins India Ink, diluted 1:1,000 in

Eagles plus 20% serum, was added to the flying coverslip tubes. Coverslips were removed 1.5 hours after carbon was applied, washed in either Hanks' or Eagles, to remove all the extracellular carbon, air dryed, and stained in May-Gruenwald Giemsa stain. These stained coverslips were observed microscopically and scored for the degree of phagocytic activity which had occurred. Seven or more selected areas were counted on each coverslip, scoring each cell that contained carbon. The percent phagocytosis of the coverslip was calculated and the average of 2 coverslips for each individual testing period and system was recorded as the percent phagocytosis for that particular system.

Bacteria

(1) Staphylococcus aureus

Staphylococcus aureus used in this study was cultured on nutrient agar. Five percent formalin was added to the cultures for 48 hours to kill the bacteria. The bacteria were then washed 4 times in 0.85% saline to remove all traces of formalin, transferred to a sterile spectrophotometric tube, and adjusted to 20% transmittance at a wavelength of 600 nanometers on the Bausch and Lomb Spectronic-20. Serial 10 fold dilutions were made and plated onto nutrient agar plates, incubated 24 to 48 hours, and the colonies counted to determine the precise viable count.

(2) Live Bacteria

The attenuated strain of Francisella tularensis, Jap, used in this study was originally obtained from the Communicable Disease Center, Atlanta, Georgia. The strain was cultured on glucose cysteine blood agar (GCBA) (Downs et al., 1947), and grown 24 hours prior to experimentation. A

24 hour culture of the bacteria was grown on GCBA, suspended in 0.85% saline, transferred to a sterile spectrophotometric tube, and adjusted to 20% transmittance at a wavelength of 600 nanometers on the Spectronic-20. Serial 10 fold dilutions were made and plated onto GCBA, incubated 72 to 96 hours, and the colonies counted to determine the viable count.

Agglutination Test

Concanavalin A (Con A) activity was assessed and standardized by an agglutination test using Sheep Red Blood Cells. Batches of the Con A used were found to agglutinate Sheep Red Blood Cells through a concentration of 3.9μg per ml.

Injection Procedure

(1) Mice were injected intraperitoneally with 0.5 ml of Con A at a concentration of 100μg per ml and the peritoneal exudate harvested 4 days later. Control mice were injected intraperitoneally with 0.5 ml of Eagles.
(2) Rats were injected intraperitoneally with 5.0 ml of Con A at a concentration of 100μg per ml and the peritoneal cells removed 4 days later. Control rats were injected intraperitoneally with 5.0 ml of Eagles.
(3) Ten ml of Con A at a concentration of 100μg per ml were injected intraperitoneally in rabbits and the cell exudate harvested 4 days later. Control rabbits were injected intraperitoneally with oil.

Viable Bacterial Infecting Dose

Peritoneal macrophages were harvested, counted and adjusted with Hanks' BSS to a cell concentration of 1×10^6 per ml. The cell suspension

was infected with F. tularensis, Jap strain, at a ratio of 5 bacteria per cell, planted in flying coverslip tubes and allowed to incubate at 37°C.

Viable Count Studies

Rabbit peritoneal macrophages were harvested, counted, adjusted with Hanks' to a cell concentration of 1 X 10^6 per ml. After cell attachment to glass the Hanks' was replaced with Eagles MEM plus 20% bovine serum. The inoculating dose of F. tularensis, Jap strain, was 10^5 organisms per ml. The infected cells were planted in flying coverslip tubes in 1 ml aliquots. The study was followed by plating onto GCBA, and staining with May-Gruenwald Giemsa at 0, 12, 24, 48, 72, and 96 hours.

At designated times, a representative coverslip from the system was crushed and thoroughly ground which disrupted the cells and released the bacteria into the media. A glass rod formed to fit the bottom of the flying coverslip tube was used for the mechanical means of disruption. Serial 10 fold dilutions were made of the media in 0.85% saline and 0.1 ml quantities of the appropriate dilutions were plated onto duplicate GCBA plates. The inoculum was spread over the surface of the GCBA plates with a glass spreader, the plates inverted, and incubated at 37°C for 72 to 96 hours and counted. Previous studies had shown that F. tularensis did not survive in cell culture media alone (Ostander 1966; Miller 1969) so that viable plate counts were thus considered to indicate viable intracellular organisms.

RESULTS

Species Uptake of Carbon Particles

When carbon particles were added to macrophages that had been in tissue culture 1.5 hours phagocytosis of the particles were observed in all four species. Rabbit peritoneal macrophage control cells maintained in tissue culture for 72 hours appeared well rounded and showed an extensive cytoplasm with some granulation (Fig. 1). Carbon particle uptake by rabbit macrophages, shown in Figure 2, demonstrated that by this time period most of the cells were phagocytic with numerous particles ingested by each phagocytic cell. It was also observed that the percent of phagocytosis increased with the time the macrophages were in tissue culture for all four species. Rabbit macrophages (Fig. 3) had the largest increase in percent phagocytosis versus cell age in culture while hamster macrophages increased the least. Mouse macrophages appeared to acquire increased phagocytic activity of carbon particles before that of other species tested. All four species showed 80-95 percent phagocytosis of the carbon particles by 72 hours.

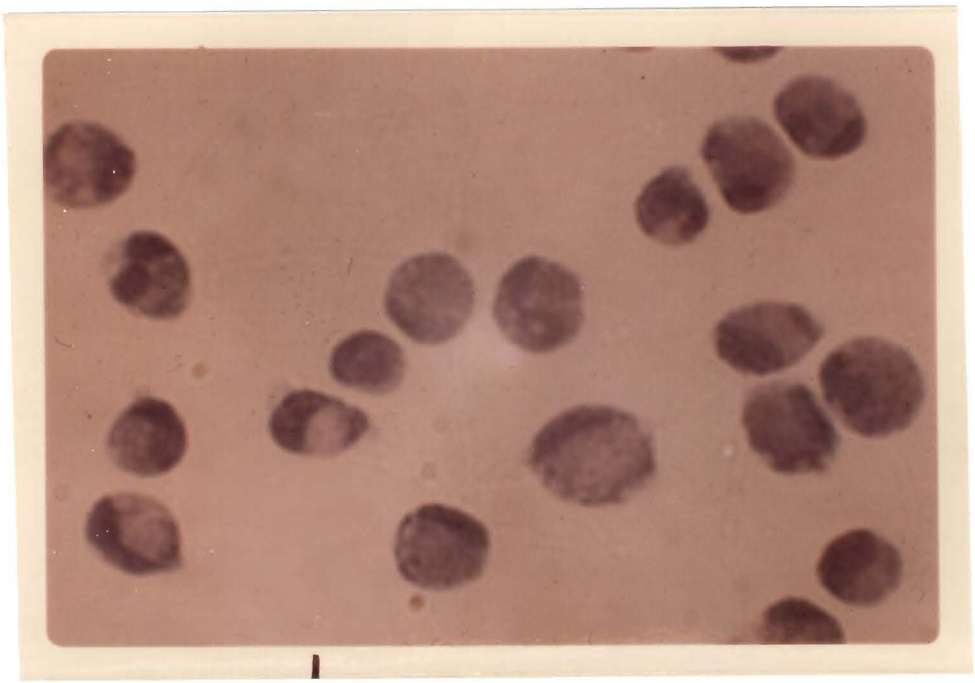
Influence of Concanavalin A Concentration on the Uptake of Carbon Particles

Since little was known about the events that occurred initially at the cell surface prior to and during the phagocytic act a chemical, Concanavalin A, known to act on the cell surface, was employed to determine some of these events. Concanavalin A was known to react with certain carbohydrates in the cell membrane.

Since rabbit macrophages had the largest increase in percent phagocytosis

Figure 1: Control culture of rabbit peritoneal macrophages in tissue culture for seventy-two hours (675X).

Figure 2: Rabbit peritoneal macrophages in tissue culture for seventy-two hours showing carbon particle uptake (675X).



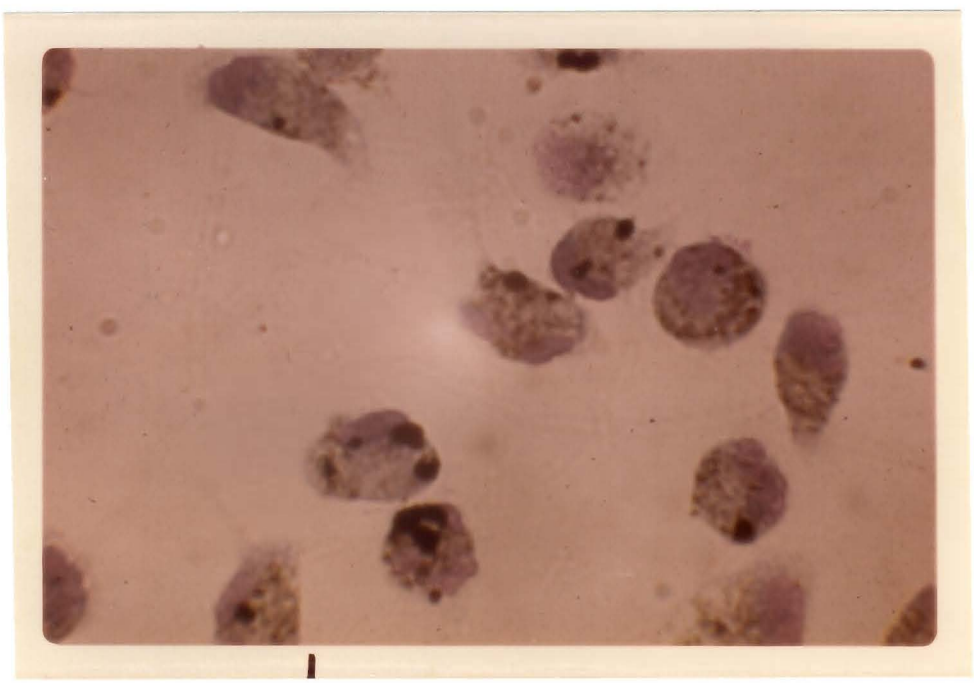
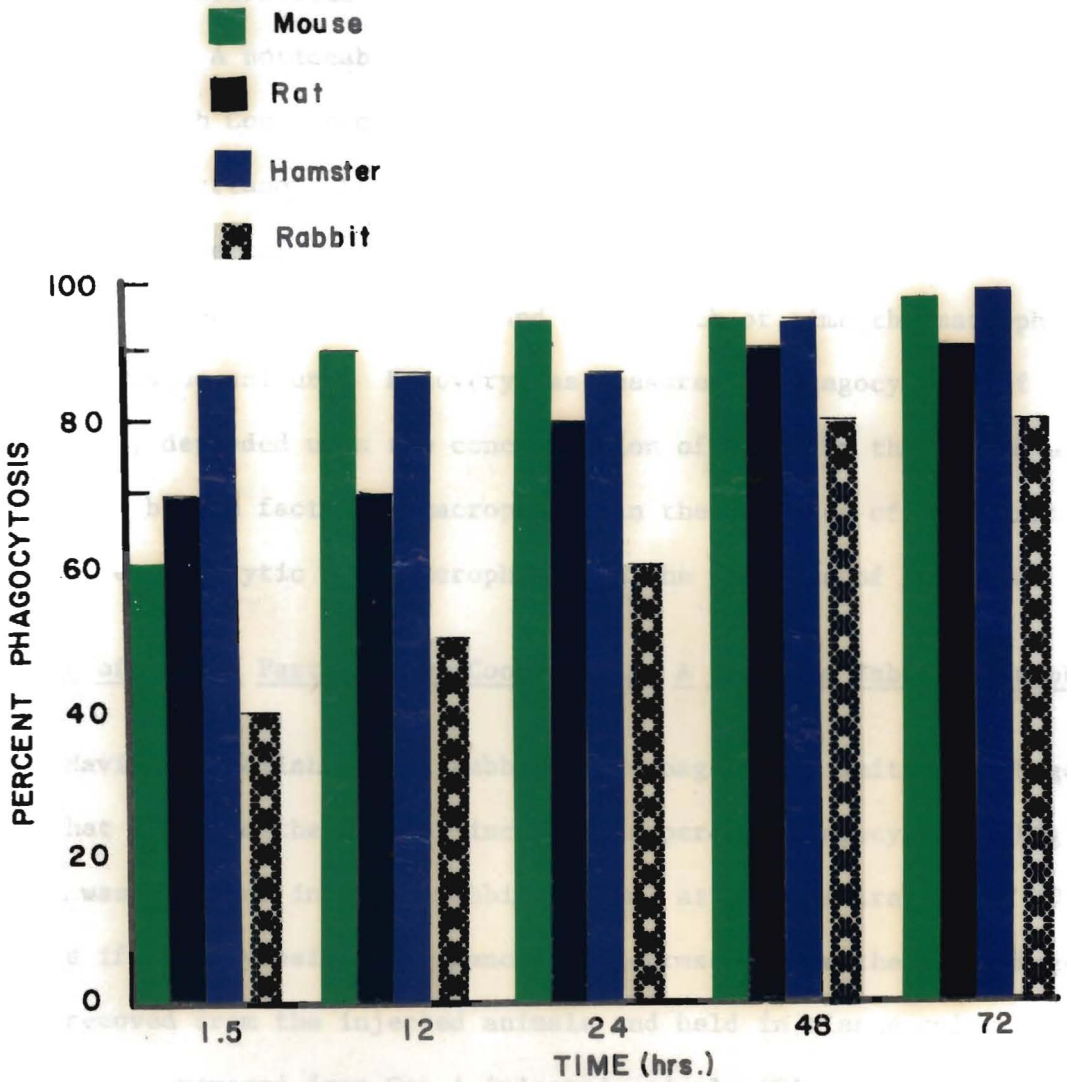


Figure 3: Comparative study of carbon particle uptake by four mammalian species.


Mouse
Rat
Hamster
Rabbit
100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
1.5
12
24
48
72
TIME (hrs.)

versus cell age in culture (Fig. 3), this species was chosen to determine what the concentration of Concanavalin A added to the tissue culture medium would have on phagocytosis. Figure 4 shows the effect of concentration of Concanavalin A on the uptake of carbon particles by rabbit macrophages. A noticeable suppression of phagocytic action towards carbon particles with Con A occurred in the first 24 hours. However, as the time in culture increased, there was a recovery of phagocytic activity suggesting a change in the cell surface. The depression of phagocytic action depended upon the concentration of Con A and the length of time the macrophages were in tissue culture. Recovery, as measured by phagocytosis of carbon particles, depended upon the concentration of Con A in the medium as evidenced by the fact that macrophages in the presence of 15.6 μg/ml Con A were more phagocytic than macrophages in the presence of 500 μg/ml Con A.

Uptake of Carbon Particles by Concanavalin A Injected Rabbit Macrophages

Having established that rabbit macrophages were initially phagocytic and that they had the largest increase in percent phagocytosis (Fig. 3) Con A was injected into the rabbits (10 ml at a concentration of 100 ug/ml) to see if phagocytosis was enhanced or depressed when the macrophages were removed from the injected animals and held in tissue culture. Macrophages removed from Con A injected animals (Fig. 5) were more phagocytic than oil stimulated macrophages from the same species. Thus, there was a stimulation of phagocytic action by Con A injected macrophages over and above that obtained with oil, and in contrast to the suppression of phagocytosis when Con A was added in vitro to the rabbit macrophages.

Figure 4: The result of different concentrations of Con A on the uptake of carbon particles by rabbit peritoneal macrophages *in vitro*.

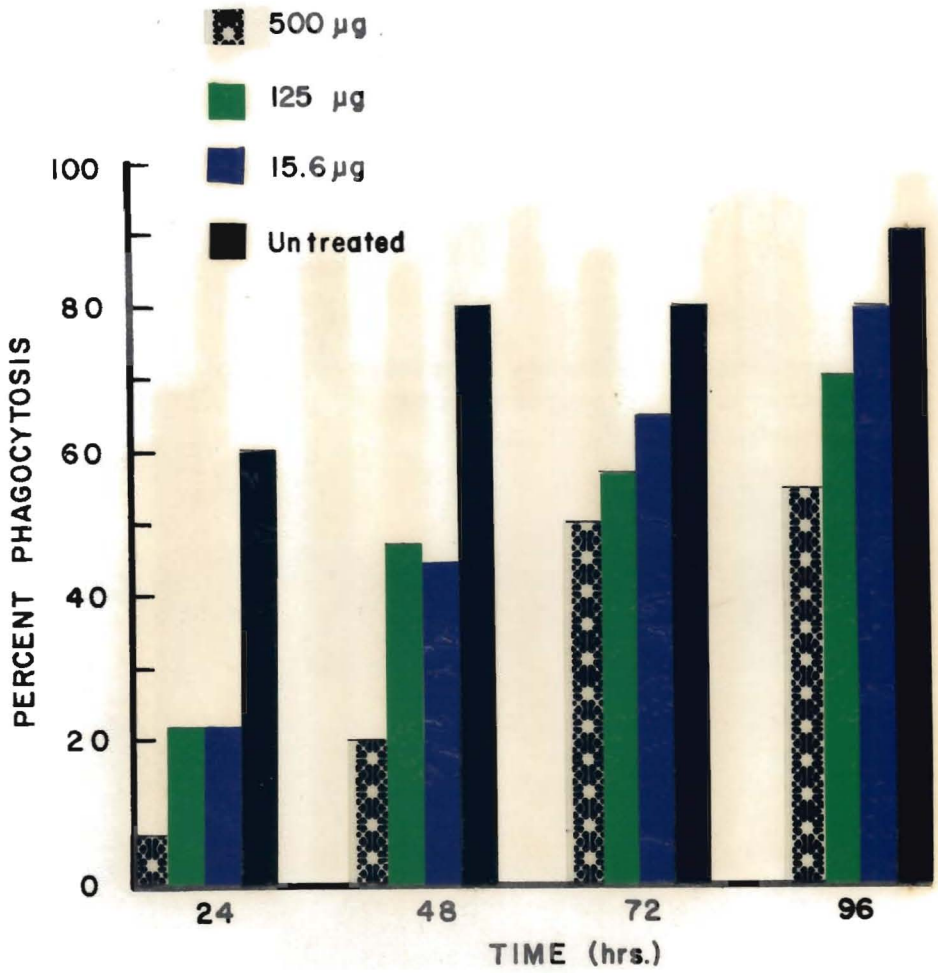
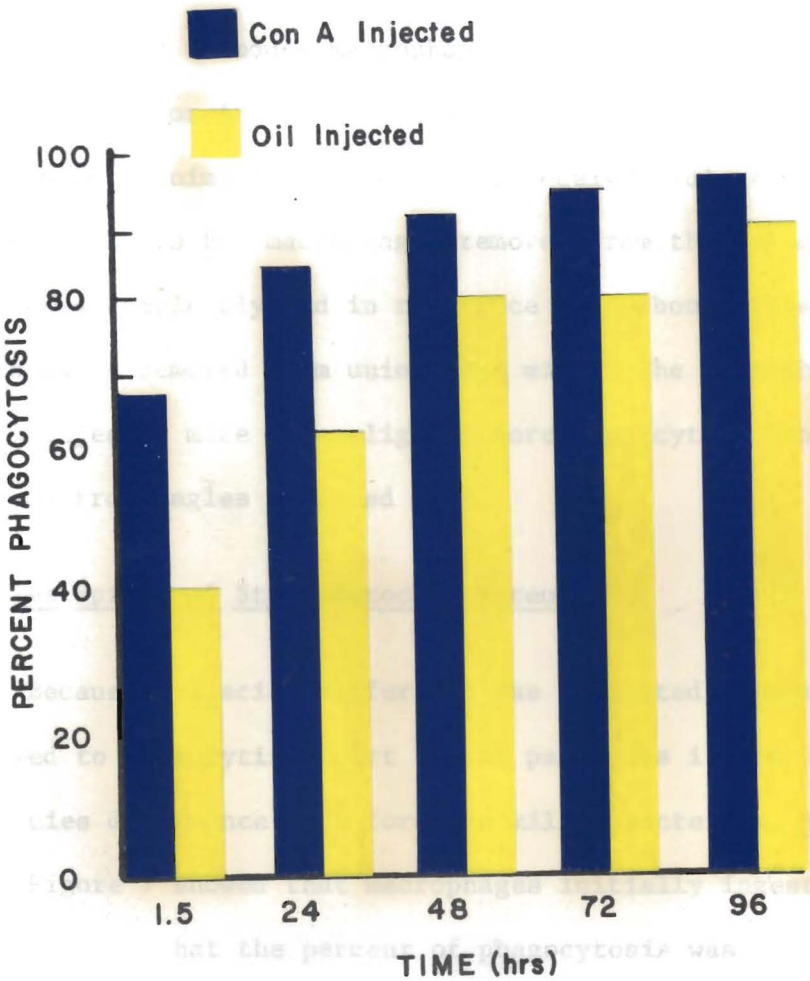

500 µg
125 µg
15.6 µg
Untreated
100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
24
48
72
96
TIME (hrs.)

Figure 5: Phagocytosis of carbon particles *in vitro* by rabbit peritoneal macrophages removed from Con A and Eagles injected rabbits.


Con A Injected
Oil Injected
100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
1.5
24
48
72
96
TIME (hrs)

Uptake of Carbon Particles by Concanavalin A Injected Mouse Macrophages

Because mouse macrophages appeared to acquire increased phagocytic uptake of carbon particles before that of the other species tested (Fig. 3) it seemed important to determine the effect Con A injected into mice would have on the phagocytosis of carbon particles. The data from Figure 6 indicated that in mouse macrophages there was a depression of both cells removed from Con A injected mice and Eagles injected mice, over cells removed from animals that had never received injections of any kind (Fig. 3). By twelve hours the macrophages removed from the injected animals had recovered completely and in reference to carbon uptake corresponded to macrophages removed from uninjected mice. The macrophages removed from Con A injected mice were slightly more phagocytic than those macrophages removed from Eagles injected mice.

Species Uptake of Staphylococcus aureus

Because a species difference was indicated when macrophages were allowed to phagocytize inert carbon particles it was decided to test for a species difference of a formalin killed bacterium, Staphylococcus aureus.

Figure 7 showed that macrophages initially ingested the killed bacteria and that the percent of phagocytosis was increased with the time the cells were maintained in culture as had occurred with carbon particles (Fig. 3). Mouse macrophages appeared to have the largest increase in percent phagocytosis versus cell age in culture while hamster macrophages increased the least. Rat macrophages were able to ingest the bacteria more readily than the other species. By 96 hours all three species tested were only 40-55 percent phagocytic of bacteria as compared to the same

Figure 6: Phagocytosis of carbon particles *in vitro* by mouse macrophages removed from Con A and Eagles injected mice.

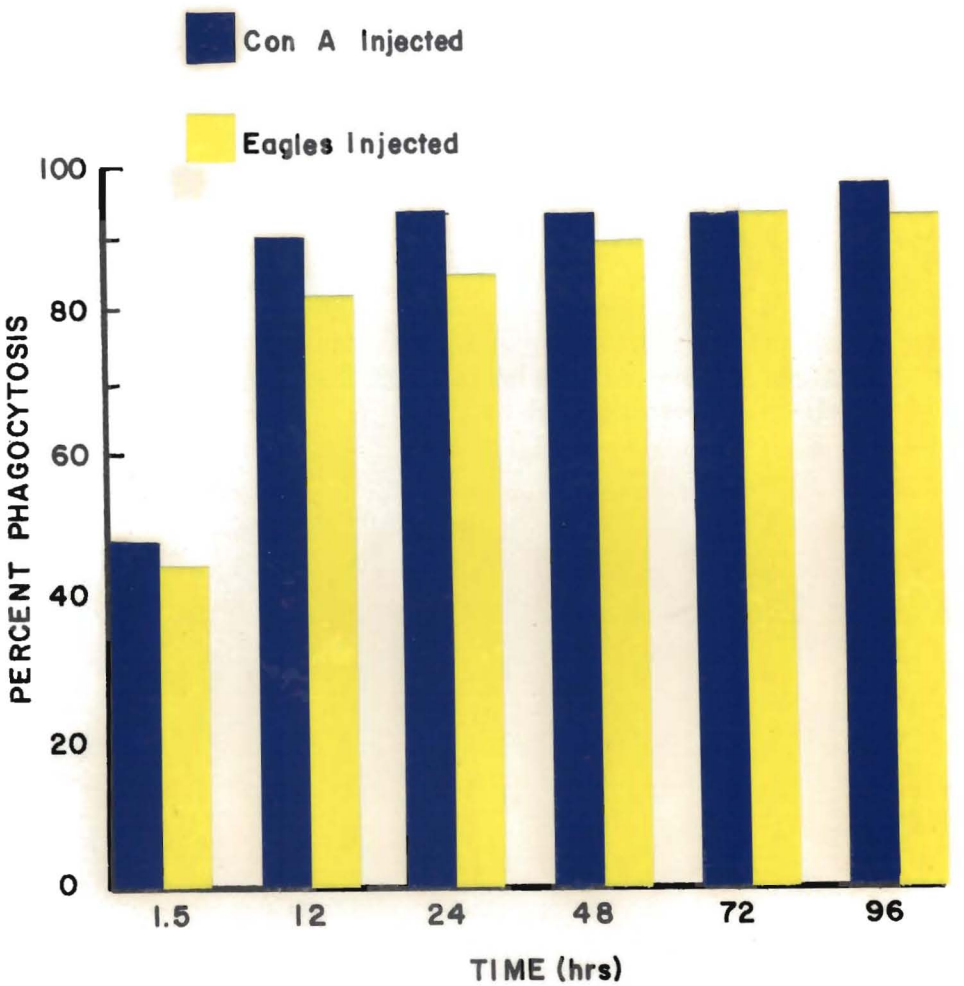
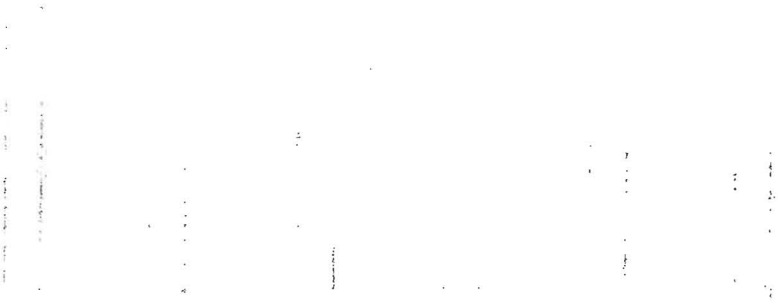
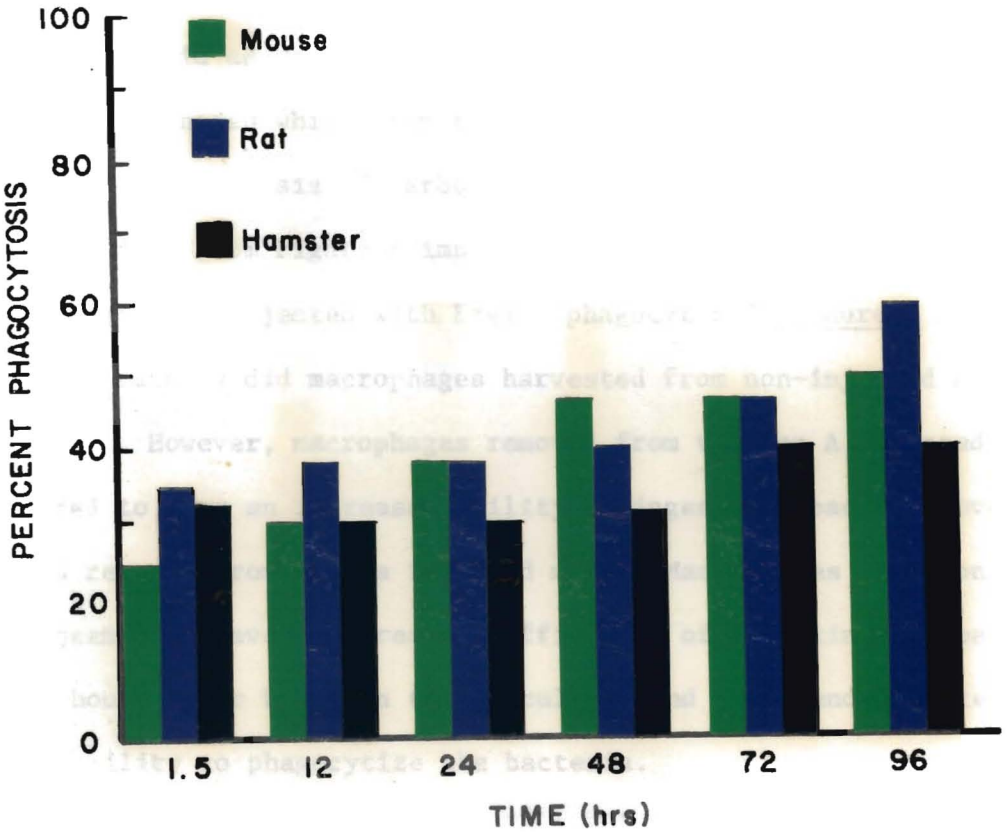

Con A Injected
Eagles Injected
PERCENT PHAGOCYTOSIS
100
80
60
40
20
0
1.5
12
24
48
72
96
TIME (hrs)

Figure 7: A comparison of the uptake of Staphylococcus aureus by three mammalian species.




100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
Mouse
Rat
Hamster
1.5
12
24
48
72
96
TIME (hrs)

three species which were over 90 percent phagocytic of carbon particles at the same time period.

Uptake of S. aureus by Concanavalin A Stimulated Mouse Macrophages

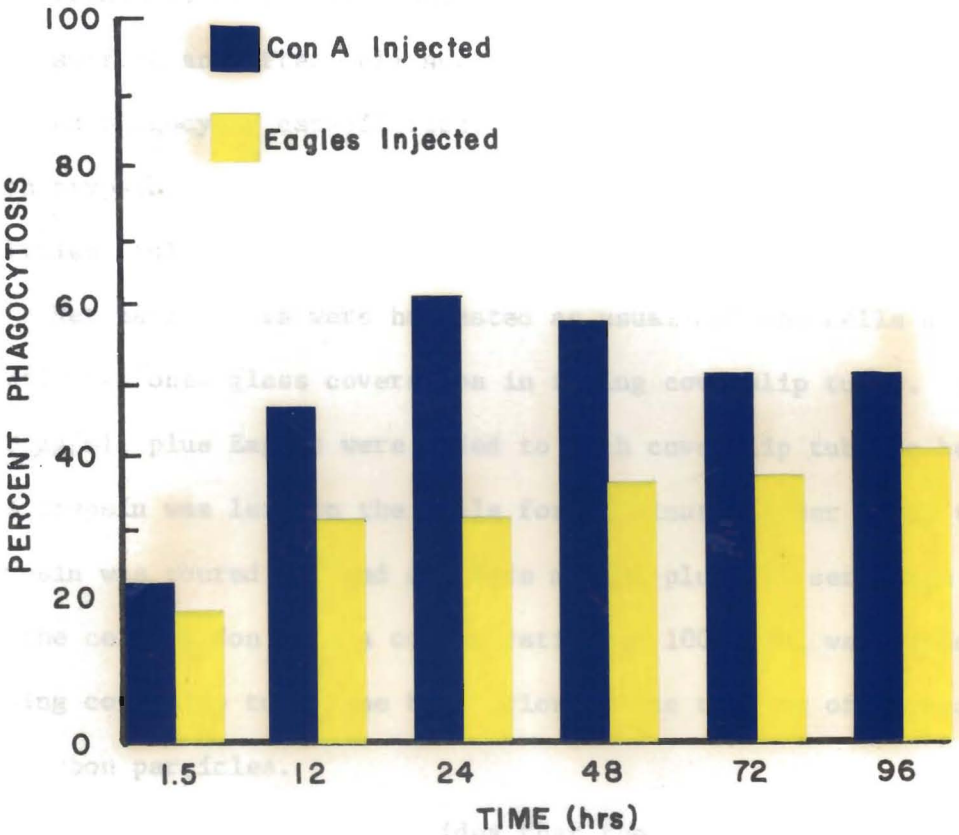
Since mouse macrophages appeared to acquire the largest increase in percent phagocytosis (Fig. 7) it seemed important to consider what effects Con A injected into mice might have on the phagocytosis of killed bacteria, i.e., would the mouse macrophages show a depression of phagocytosis of killed bacteria as they did carbon particles or would they act as the rabbit macrophages which when removed from Con A injected rabbits stimulated the phagocytosis of carbon particles?

The data from Figure 8 implies that mouse macrophages removed from mice previously injected with Eagles phagocytized S. aureus at approximately the same rate as did macrophages harvested from non-injected animals (Fig. 7). However, macrophages removed from the Con A injected mice appeared to show an increased ability to ingest the bacteria over macrophages removed from Eagles injected mice. Macrophages from Con A injected mice seemed to have the greatest efficiency of ingesting the bacteria at 24-48 hours after being in tissue culture and then tended to level off in their ability to phagocytize the bacteria.

Uptake of S. aureus by Concanavalin A Stimulated Rat Macrophages

Untreated rat macrophages ingested percentagewise the greatest number of killed bacteria (Fig. 7). Therefore, it seemed relevant to find out whether or not the phagocytic action of rat macrophages could be further enhanced by the injection of Con A into the animals.

Figure 8: Uptake of Staphylococcus aureus in vitro by mouse macrophages removed from Con A and Eagles injected mice.


100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
Con A Injected
Eagles Injected
1.5
12
24
48
72
96
TIME (hrs)

Macrophages removed from Con A injected rats (Fig. 9) appeared to be stimulated above and ingest more bacteria than do macrophages from Eagles injected rats. However, the stimulatory effect lasted only through 48 hours after which time the macrophages from both systems phagocytized the bacteria at approximately the same rate.

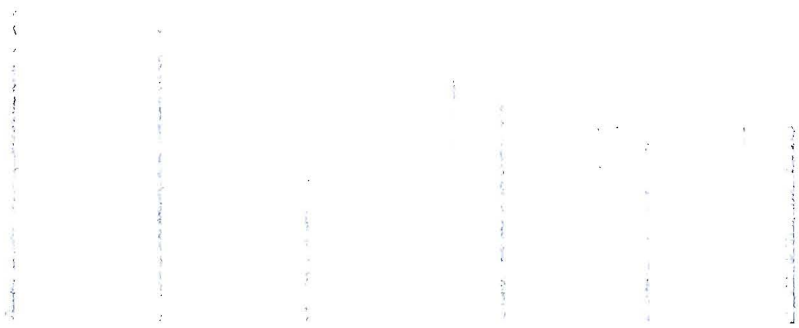
Carbon Particle Uptake by Rat Macrophages After Trypsin Treatment

Treatment of polymorphonuclear leucocytes with trypsin modified the cell surface and after cell surface repair these cells demonstrated increased phagocytic capabilities. Therefore rat macrophages were treated with trypsin and trypsin and Con A in hopes that their phagocytic capabilities could be further enhanced.

Rat macrophages were harvested as usual and the cells allowed to settle out onto glass coverslips in flying coverslip tubes. Trypsin, 500 µg/ml, plus Eagles were added to each coverslip tube to be tested. The trypsin was left on the cells for 45 minutes after which time the trypsin was poured off and complete medium plus 20% serum was added back to the cells. Con A in a concentration of 100 µg/ml was added to the flying coverslip tubes one hour prior to the testing of phagocytic uptake of carbon particles.

Figure 10 supported the idea that the strong trypsin treatment changed the cell surface and little uptake of carbon occurred initially. By twelve hours the cell surface was repaired and a large increase of phagocytosis over and above the phagocytosis of carbon particles by untreated rat macrophages was observed. This graph also seemed to indicate that Con A added to the culture medium after trypsin treatment in some way aided the

Figure 9: Phagocytosis of Staphylococcus aureus in vitro by rat macrophages removed from Con A and Eagles injected rats.



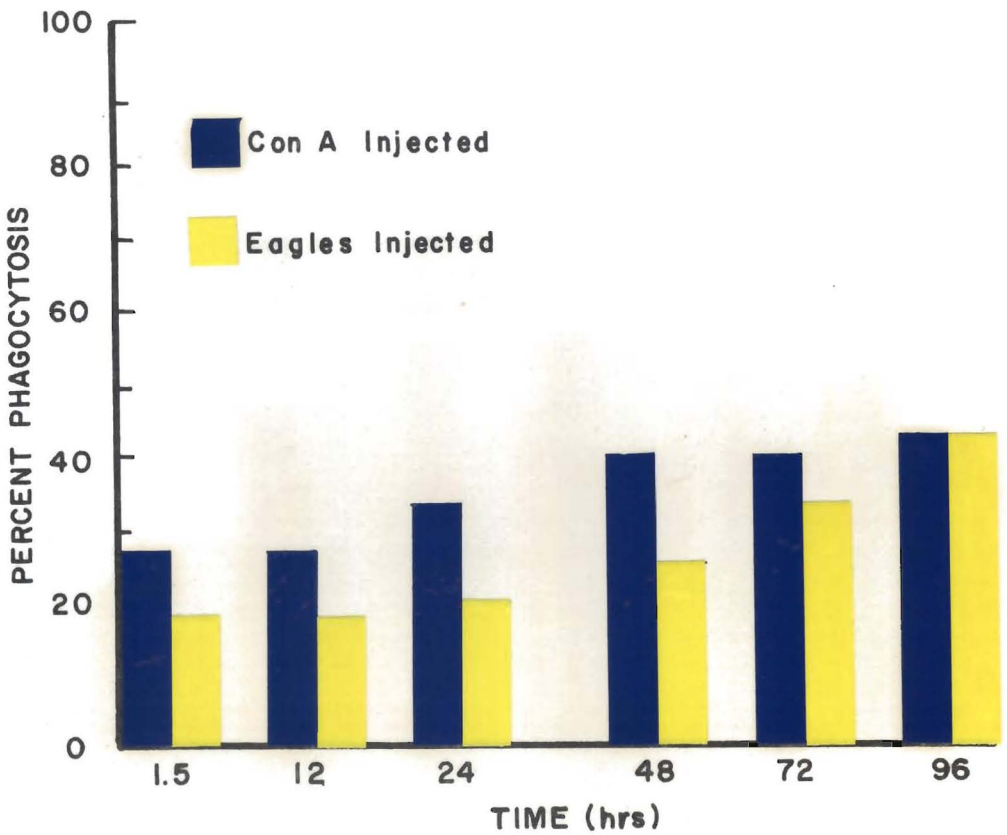
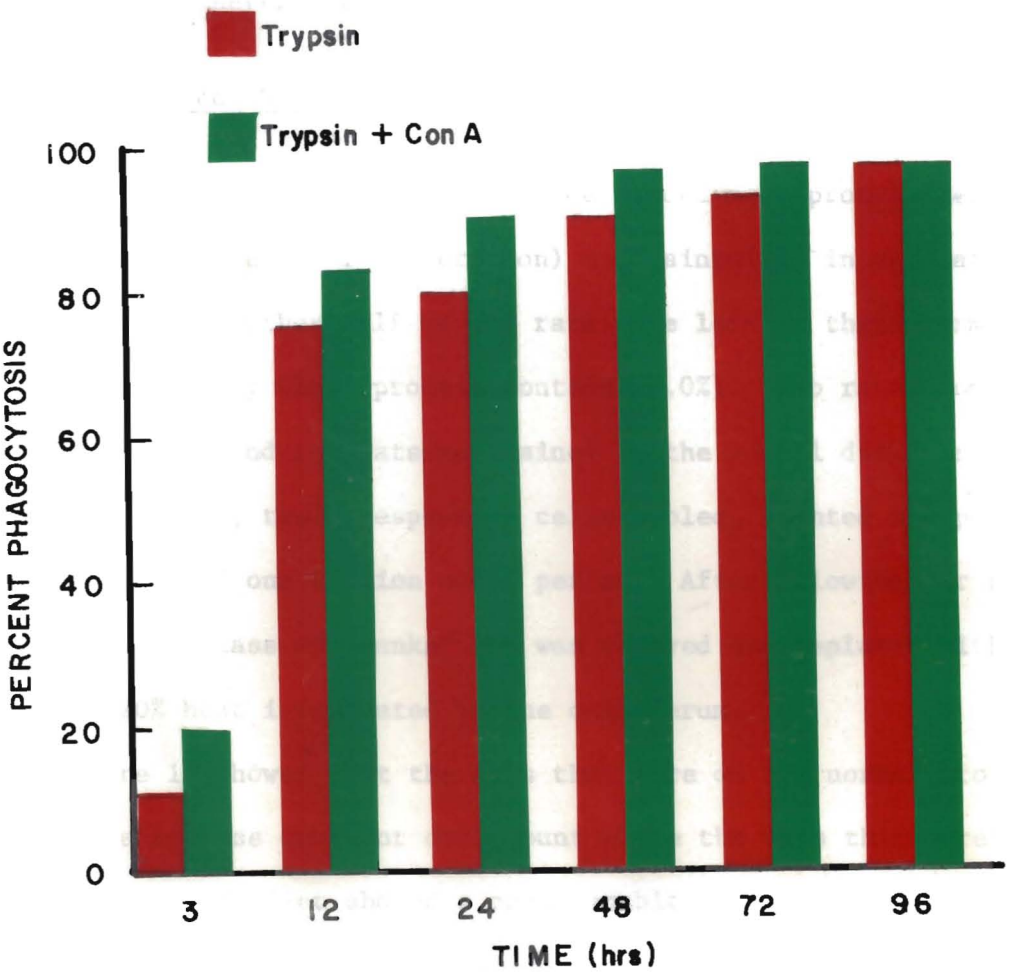

100
80
60
40
20
0
PERCENT PHAGOCYTOSIS
Con A Injected
Eagles Injected
1.5
12
24
48
72
96
TIME (hrs)

Figure 10: Uptake of carbon particles by rat macrophages after treatment with trypsin.


Trypsin
Trypsin + Con A
PERCENT PHAGOCYTOSIS
100
80
60
40
20
0
3
12
24
48
72
96
TIME (hrs)

repair of the cell surface since macrophages treated with Con A recovered faster and showed increased phagocytic ability. By 48 hours the cell surface repair appeared complete as there was little difference in phagocytosis from then on. An enhancement of carbon particle uptake by trypsin treated as well as trypsin plus Con A treated cells was indicated over cells that had never been treated (Fig. 3).

Protein Deficient Diet and the Number of Peritoneal Exudate Cells

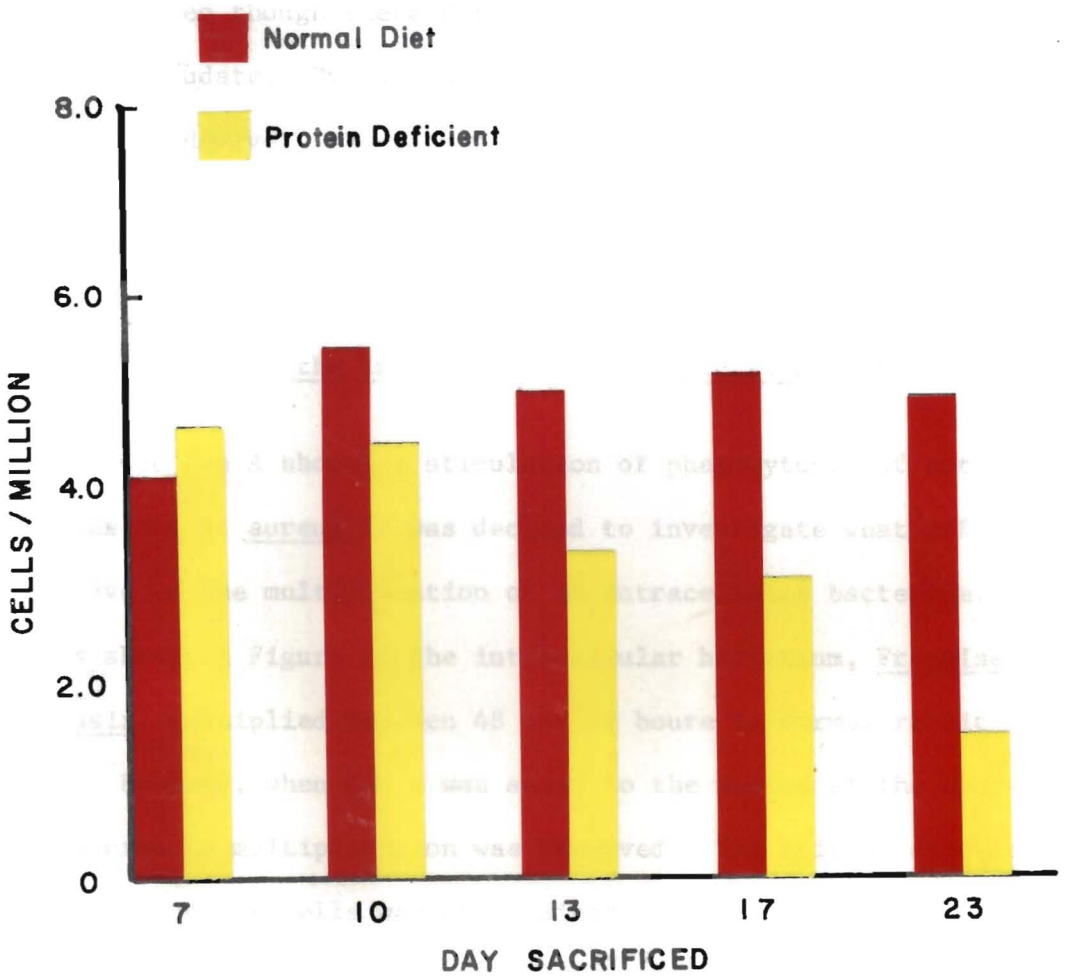
Half of the rats to be tested were placed on a protein deficient diet (Nutritional Biochemical Corporation) and maintained in separate housing facilities. The other half of the rats were left on their normal diet (Purina Laboratory Chow, protein content 23.0%). Two rats from the protein deficient group and two rats maintained on the normal diet were sacrificed at the same time, their respective cells pooled, counted and planted at a concentration of one million cells per ml. After allowing for cell attachment to glass the Hanks' BSS was removed and replaced with Eagles MEM plus 20% heat inactivated bovine calf serum.

Figure 11 showed that the rats that were on the normal protein diet had a more or less constant cell count while the rats that were on the protein deficient diet showed a considerable loss in the number of exudate cells removed from the peritoneal cavity. The first large drop in cell number occurred on the 13th day the animals were on the protein deficient diet and the largest decrease in cell number was noted by the 23rd day.

Recovery of Protein Deficient Rat Macrophages

After the cells were planted in complete medium their phagocytic

Figure 11: Number of cells removed from the rat peritoneal cavity modified by a protein deficient diet.


Normal Diet
Protein Deficient
8.0
6.0
4.0
2.0
0
CELLS / MILLION
7
10
13
17
23
DAY SACRIFICED

capability was assessed by the addition of carbon particles at the correct time period. Figure 12 showed that initially the protein deficient macrophages were more phagocytic than the macrophages from the rats on the normal diet. It was also noted that macrophages removed from animals that had been on the protein deficient diet for 23 days were initially the most phagocytic even though these rats had the fewest number of cells in the peritoneal exudate. By 24 hours the cells appeared to have recovered completely. However, the macrophages removed from protein deficient animals in all cases were more phagocytic than macrophages removed from animals on the normal diet.

Effects of Con A on the Intracellular Multiplication of F. tularensis

Because Con A showed a stimulation of phagocytosis of both carbon particles and S. aureus it was decided to investigate what effect Con A would have on the multiplication of an intracellular bacterium.

As shown in Figure 13 the intracellular bacterium, Francisella tularensis, multiplied between 48 and 72 hours in normal rabbit macrophages. However, when Con A was added to the medium at the same time as the bacteria no multiplication was observed. The initial drop in viable bacteria in normal cells was unexplained. A slight increase occurred at 96 hours in the Con A system. From microscopic observations of stained slides at the appropriate time intervals the bacteria from the Con A system was found to be phagocytized by the peritoneal macrophages.

Figure 12: In vitro recovery of rat macrophages from rats maintained on both normal and protein deficient diets.

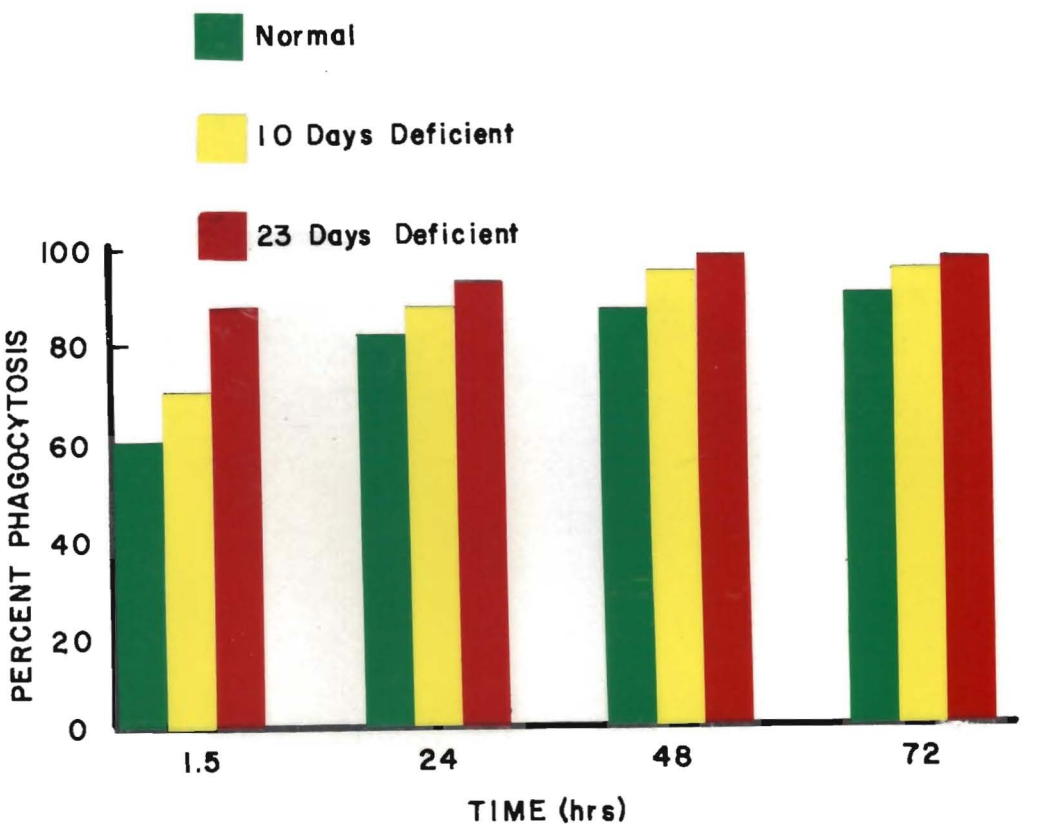
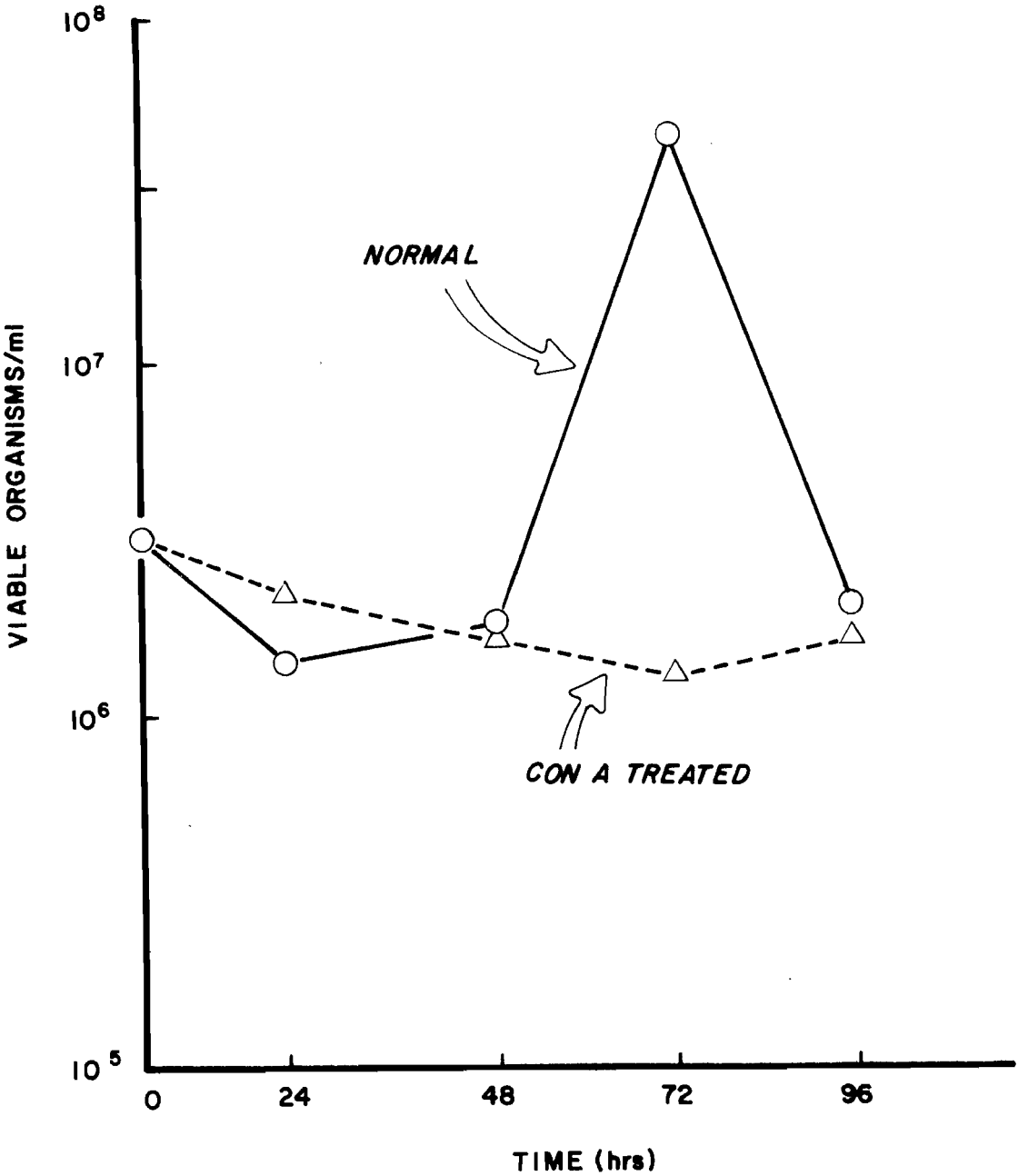

Normal
10 Days Deficient
23 Days Deficient
PERCENT PHAGOCYTOSIS
100
80
60
40
20
0
1.5
24
48
72
TIME (hrs)

Figure 13: The multiplication of Francisella tularensis in rabbit peritoneal macrophages removed from normal rabbits and treated with Con A in vitro.


10^8
10^7
10^6
10^5
VIABLE ORGANISMS/ml
NORMAL
CON A TREATED
0
24
48
72
96
TIME (hrs)

DISCUSSION

A difference exists between species in their ability to fight infection since some animals are more resistant to disease than others. One important mechanism in fighting diseases is the phagocytosis of the invading organism. Because differences in species susceptibility occur, the question was asked if this difference might be reflected in the species ability of phagocytizing the invading organism.

One of the simplest ways to measure phagocytosis is by the uptake of inert carbon particles by peritoneal macrophages. Thus, an investigation of inert carbon particle uptake by peritoneal macrophages from various species was undertaken. A species difference in the uptake of carbon particles was indicated with rabbit macrophages showing the largest increase in percent phagocytosis. Hamster macrophages showed the least increase. Initially, hamster macrophages were able to ingest more carbon particles than other species tested and they maintained a heightened ability as compared to other species as the in vitro conditions did not significantly alter their phagocytic capabilities. Mouse macrophages appeared to acquire increased phagocytic activity sooner than other species. That in vitro conditions of culture seemed to activate mouse macrophages before those of other species examined was evident from the shorter time required for them to become highly phagocytic.

Since several plant lectins have been shown to modify the cell surface it was decided that one of these lectins might be used to modify the surface of macrophages to determine if the phagocytic abilities of the various macrophages were concomitantly altered. Rabbit macrophages

were used to investigate the effect of varying concentrations of Con A upon phagocytosis in a tissue culture medium. Initially a noticeable suppression of uptake of carbon occurred. However, with increased time the macrophages apparently recovered their phagocytic ability. Berlin (1972) reported that Con A inhibited phagocytosis of inert polyvinyltoluene particles by rabbit polymorphonuclear leucocytes and that another membrane function, the transport of non-electrolytes (adenine and nucleoside transport) was also unaffected. At a concentration of 100 µg/ml he found that none of the beads were taken into the cell; rather they appeared to form rosettes at the cell periphery and at 4°C Con A blocked virtually all association of beads with cells. However, in our studies rabbit monocytes phagocytized carbon particles at all concentrations utilized. Rabbit monocytes apparently differ from rabbit polymorphonuclear leucocytes which are unable to phagocytize the beads in the presence of Con A. Berlin (1972) suggested that either Con A prevented the adsorption of particles to specific phagocytic sites or that it prevented internalization of the membrane subsequent to particle-site interaction. Since our investigation showed carbon particle uptake at all concentrations and since Con A does not prevent internalization of the membrane (electron microscopic studies by Barat, 1973, showed Con A lining the phagocytic vacuole) it appears that macrophages are not similar to polymorphonuclear leucocytes in their mode of action of phagocytosis as tested by treatment with Con A.

A previous investigator (Jennings, 1970) reported highly phagocytic macrophages after injection of Phytohemagglutinin (PHA) intraperitoneally in mice. However, other workers (Robbins, 1964; Lozzio, 1967; and Panzetta, 1973) found PHA injected intraperitoneally in mice depressed

phagocytosis. Because of these conflicting reports of this lectin's action when injected into animals, and the animals apparent change in phagocytic capabilities, Con A was injected intraperitoneally in rabbits. Our study showed an enhanced phagocytic ability of macrophages removed from Con A injected rabbits over macrophages removed from oil stimulated rabbits. Con A injected animals may have activated their macrophages more than the oil stimulated macrophages and hence accounted for their increased phagocytic ability. This factor of activation or stimulation may well depend upon the dose injected, the period of adsorption from the peritoneal cavity, and also the species involved.

A further investigation of species difference was shown when mice were injected with Con A and Eagles. Both macrophage systems showed a depression of phagocytosis of carbon particles when compared to mouse macrophages that had never been treated. This difference could be related to the dose injected and the period of time before the macrophages were removed from the animal, since the macrophages appeared to be quickly stimulated to a high degree of phagocytosis after a short time in culture.

Because in the investigation of phagocytosis a species difference was found with inert carbon particles, the question was asked if a species difference also existed in the phagocytosis of bacteria. Our results showed that macrophages removed from the animals were initially phagocytic although the percent of phagocytosis was significantly smaller for the killed bacteria than that of carbon particles. Hirt (1973) reported that mouse and guinea pig macrophages, freshly harvested and suspended in either heterologous or homologous serum, phagocytized S. aureus inefficiently. After the bacteria were incubated for two hours with the

macrophages 90% were cell associated but phagocytosis did not occur. Our findings show an increased phagocytic capability with the time maintained in tissue culture. This finding is also consistent with Hirt (1973), who showed that macrophages cultured in vitro for two days were able to kill S. aureus as efficiently as macrophages from BCG-immunized animals. This enhanced bactericidal capacity appeared to be a consequence of macrophage maturation in vitro. This consistent increase in percent phagocytosis with time in culture suggests that macrophages are stimulated, since active spreading leads to a larger surface area and allows more surface receptors to become exposed. Rat macrophages were able to ingest the bacteria better than other species tested suggesting that rats are better able to combat the bacterial infection.

Since a species difference of killed bacteria was indicated relative to phagocytosis, mice and rats were injected with Con A in an effort to enhance their phagocytic uptake of bacteria. Our investigations showed that macrophages removed from Con A stimulated animals showed an enhanced ability to phagocytize the bacteria over animals that were injected with Eagles. Van Oss (1972) found that bacteria with a contact angle lower than that of the macrophages were phagocytized to a much lesser extent and tended to be more pathogenic than bacteria with a higher contact angle. It is possible that Con A injected into animals stimulates their macrophages when they are removed and not only is their surface area larger but the contact angle may be less between the macrophages and the bacteria thus allowing for increased phagocytosis.

Treatment of cells with proteolytic enzymes has been shown to enhance the uptake of particulate materials. Proteolytic enzymes induce changes

in the surface structure similar to that brought about by transformation. In this study it was found that after trypsin treatment little phagocytosis occurred initially. The cell surface is completely repaired six hours after trypsin treatment (Burger, 1971). Our results imply that after trypsin treatment Con A apparently aided the repair of the cell surface since trypsinized macrophages treated with Con A recovered faster and showed increased uptake of carbon particles.

Due to the present increased interest in protein nutrition we also investigated the effects of a nutritional deficiency upon phagocytosis. Our results indicated that the total number of cells removed from the peritoneal exudate decreased with the number of days the animals were maintained on a protein deficient diet. The protein deficient cells at 1.5 hours in culture were more phagocytic than the normal macrophages. Removal of macrophages from a low protein environment to a high protein environment resulted in an apparent enhanced phagocytic ability. However, this enhancement may have resulted from the high content of serum in the media. By 24 hours the macrophages removed from protein deficient rats were more phagocytic than those of normal rats. Macrophages deficient in protein for 23 days were more phagocytic through the entire time phagocytosis was measured than normal macrophages. Whether these protein deficient macrophages were activated is not known.

Because Con A stimulated the phagocytic capabilities of macrophages from various species and because an increase in phagocytosis of both inert carbon particles and S. aureus was observed an additional experiment was designed to monitor the effects of Con A on the multiplication of Francisella tularensis, a bacteria that multiplies intracellularly. It

was found that multiplication in normal rabbit peritoneal macrophages occurred between 48 and 72 hours after infection in tissue culture. When Con A was added to the medium at the same time as the bacteria no multiplication of the bacteria was observed. Klun (1973) showed that when PHA was added to supernatant fluids from cultures of lymphocytes from non-immunized and immunized mice growth of intracellular virulent tubercle bacilli occurred. However, if Con A was added to lymphocytes from animals immunized against mycobacteria both supernatant fluids brought about the inhibition of the intracellular growth of the tubercle bacilli (Klun, 1973). In our studies a slight increase in the number of bacteria in the Con A system was observed at 96 hours although this rise was probably not significant as it was still less than the infecting dose. Bacteria in the Con A system were phagocytized since it had been shown previously (Ostander, 1966; Miller, 1969) that F. tularensis would not survive in the media alone which would have been indicated by a drop in the number of viable bacteria. Although Con A did prevent multiplication of the bacteria, the bacteria could be seen in phagocytic vacuoles. The bacteria were not killed as evidenced by the viable count but apparently remained in a bacteriostatic state. Barat (1973) in an electron microscopic study of normal human lymphocytes treated with Con A found that after fifteen minutes incubation at 37°C a number of positive intracytoplasmic vesicles were labeled which suggested active pinocytosis and indicated that a fraction of the external membrane was used in the formation of these intracytoplasmic membranes. Singer (1972) proposed a new membrane model which suggested that phospholipids and proteins do not have a fixed location in the membrane but move freely without requiring the synthesis

of new membrane. Thus, a possible explanation for the prevention of multiplication of F. tularensis, in the presence of Con A, was that Con A was incorporated into the phagocytic vacuole and thus would not allow, through membrane changes, fusion of the lysosome and consequent killing and digestion. Thus the bacteria could not replicate within the cell. It was also possible that after phagocytosis Con A reacted with the bacterial surface and this prevented the bacteria from multiplying.

Several mechanisms of action have recently been proposed for Con A. Nicolson (1973) proposed that the clustering of lectin sites on transformed cells may have been a result of a difference in fluidity of the transformed cell membranes which allowed disturbational perturbation induced by polyvalent lectin molecules such as Con A. This difference in fluidity of the membrane allowed clustering of surface sites and thus could explain the enhanced agglutinability of tumor cells without the use of the theories on cryptic-site masking or unmasking or the two-site hypothesis as was proposed by Inbar in 1971 (Nicolson, 1973). Changes resulting from lectins binding to specific cell receptors which are linked to an appropriate lymphocyte activation mechanism may convert a resting cell into an actively growing one. Metabolic stimulation by phagocytizable particles or surface disturbing agents such as lectins in both polymorphonuclear leucocytes and macrophages were shown to reside in the activation of a granule-bound NADPH oxidase (Romeo, 1973). Increasing this reaction rate resulted in increased respiratory activity as well as an increase in the hexose monophosphate shunt. Con A bound to macrophages stimulated their oxidative metabolism and was initiated by the activation of the granule-bound NADPH oxidase. Such metabolic stimulation could be inhibited with

sugars which compete with Con A for cell receptors (Romeo, 1973). Edelman (1972) proposed the mechanism of action of Con A to be the result of its two binding sites which function to crosslink mobile glycoprotein receptors into micropatches. These patches on the phagocytic surface may alter the surface properties of the macrophages, such as potential, and ion permeability which thus leads to a change in the activity of intracellular enzymes such as NADPH oxidase (Romeo, 1973). However, the definite mechanism or mechanisms of action of Con A are at this point still unknown.

SUMMARY

Peritoneal macrophages removed from various species of animals showed a species difference in their ability to phagocytize both inert carbon particles and a killed bacterium, Staphylococcus aureus. After removal from the peritoneal cavity, macrophages from all species showed an initial ability to engulf both types of particulate material. Rabbit macrophages had the largest increase during culture in percent phagocytosis of carbon particles while mouse macrophages had the largest increase in percent phagocytosis of killed S. aureus. Hamster macrophages showed the smallest increase in percent phagocytosis vs time in culture for both particulates.

Con A injected into the peritoneal cavity of various species prior to the removal of macrophages enhanced their phagocytic capabilities when grown in tissue culture. The uptake of both carbon and S. aureus by peritoneal macrophages was increased if the various animals received prior injections of Con A. Prior injections of Con A led to a significant enhancement of carbon particle uptake by rabbit macrophages. The ability to phagocytize carbon particles by mouse macrophages was only slightly increased by the same treatment. The ability of both species to phagocytize S. aureus was significantly increased by the treatment, however.

Placing animals on a protein deficient diet resulted in a concomitant decrease in the number of cells that could be removed from the peritoneal cavity. However, the peritoneal cells removed from the protein deficient animals displayed an increased ability to phagocytize carbon compared to cells removed from control animals.

Rabbit macrophages maintained in the presence of Con A and infected with Francisella tularensis did not allow multiplication of the bacteria, but the bacteria remained viable.

Peritoneal macrophages obtained from three mammalian species differed in their ability to phagocytize both inert carbon particles and S. aureus. Injection of Con A into the animals enhanced their phagocytic ability.

The phagocytic activities of normal cells increases with time which suggests a maturation phenomenon under in vitro conditions. Such maturation might be explained as an increase in the effective surface area of the macrophages thus allowing for more receptor sites to be exposed. One possible explanation for the non-multiplication of F. tularensis in rabbit macrophages was that some of the Con A may have been incorporated in the phagocytic vacuole which may have prevented bacterial multiplication.

LITERATURE CITED

LITERATURE CITED

Barat, N. and S. Avrameas. 1973. Surface and Intracellular Localization of Concanavalin A in Human Lymphocytes. Exptl Cell Res., 76:451.

Barrett, J.T. 1970. Textbook of Immunology. C.V. Mosby Company, St. Louis. P. 154.

Ben-Bassat, H., M. Inbar and L. Sachs. 1971. Changes in the Structural Membrane in Malignant Cell Transformation. J. Membrane Biol., 6:183.

Berlin, R.D. 1972. Effect of Concanavalin A on Phagocytosis. Nature New Biol., 235:44.

Burger, M.M. 1971. The Significance of Surface Structure Changes for Growth Control Under Crowded Conditions. In Wolstenholme, G.E.W., and J. Knight. Growth Control in Cell Cultures. Churchill Livingstone, Edinburgh. P. 45.

Downs, C.M., L.L. Coriell, H. Eigelsback, K. Plitt, D. Pinchot, and B. Owen. 1947. Studies on Tularemia. J. Immunol., 56:229.

Edelman, G.M., B.A. Cunningham, G.N. Reeke, J.W. Becker, M.J. Waxdal, and J.L. Wang. 1972. The Covalent and Three-Dimensional Structure of Concanavalin A. Proc. U.S. Nat. Acad. Sci., 69:2580.

Harris, H. 1954. Role of Chemotaxis in Inflammation. Physiol. Rev. 34:529.

Hirt, W.E. and P.F. Bonventre. 1973. Cultural, Phagocytic, and Bactericidal Characteristics of Peritoneal Macrophages. J. Reticuloendathel Soc., 13:27.

Inbar, M. and L. Sachs. 1969a. Structural Difference in Sites on the Surface Membrane of Normal and Transformed Cells. Nature, 233:710.

_____. 1969b. Interaction of the Carbohydrate-Binding Protein Concanavalin A with Normal and Transformed Cells. Proc. US Nat. Acad. Sci., 63:1418.

Inbar, M., H. Ben-Bassat, and L. Sachs. 1971. A Specific Metabolic Activity on the Surface Membrane in Malignant Cell Transformation. Proc. US Nat. Acad. Sci., 68:2748.

Jacoby, F. 1965. Macrophages. In Cells and Tissues in Culture. v. 2. Academic Press, New York. pp. 1-93.

Jennings, J.F. and L.A. Hughes. 1970. The Effect of Phytohemagglutinin on Phagocytosis in the Mouse Peritoneal Cavity. J. Reticuloendothel. Soc., 7:617.

Kapeller, M. and F. Doljanski. 1972. Agglutination of Normal and Rous Sarcoma Virus-transformed Chick Embryo Cells by Concanavalin A and Wheat Germ Agglutinin. Nature New Biol., 235:184.

Karnovsky, M.L. 1962. Metabolic Basis of Phagocytic Activity. Physiol. Rev., 42:143.

Klun, C.L., and G.P. Youmans. 1973. The Induction by Listeria monocytogenes and Plant Mitogens of Lymphocyte Supernatant Fluids Which Inhibit the Growth of Mycobacterium tuberculosis Within Macrophages In Vitro. J. Reticuloendothel. Soc., 13:275.

Lozzio, B.B. 1967. Depression of Reticuloendothelial Phagocytic Activity by Phytohemagglutinin. Proc. Soc. Exp. Biol. Med., 126:435.

Markowitz, H., D.A. Person, G.L. Gitnick, and R.E. Ritts. 1968. Immunosuppressive Activity of Concanavalin A. Science., 163:476.

Metzger, G.V. and L.J. Casarett. 1967. Some Effects of Divalent Cations on In Vitro Phagocytosis. Adv. Exp. Med. Biol., 1:163.

Miller, G.C. 1969. Further Studies on the Induction of Immune Response with Ribonuclease-Sensitive Fractions of Immune Monocytes. M. S. Thesis. Kansas State Teachers College of Emporia, Kansas.

Moore, E.G. and H.M. Temin. 1971. Lack of Correlation Between Conversion by RNA Tumor Viruses and Increased Agglutinability of Cells by Concanavalin A and Wheat Germ Agglutinin. Nature., 231:117.

Myrvik, O.N., E.S. Leake, and B. Fariss. 1961. Lysozyme Content of Alveolar and Peritoneal Macrophages from the Rabbit. J. Immunol., 86:133.

Nicolson, G.L. 1973. Temperature-dependent Mobility of Concanavalin A Sites on Tumor Cell Surfaces. Nature New Biol., 243:218.

Norman, S.J. 1973. Competitive Inhibition Between Phagocytic Particles. J. Reticuloendothel. Soc., 13:65.

North, R.J. 1966. The Localization by Electron Microscopy of Nucleoside Phosphatase Activity in Guinea Pig Phagocytic Cells. J. Ultrastruct. Res., 16:83.

_____. 1968. The Uptake of Particulate Antigens. J. Reticuloendothel. Soc., 5:203.

Oren, R., A.E. Farnham, K. Saito, E. Milofsky, and M.L. Karnovsky. 1963. Metabolic Patterns in Three Types of Phagocytizing Cells. J. Cell Biol., 17:487.

Ostrander, K. 1966. In Vitro Studies of the Interaction of Francisella tularensis with Cells of the Reticuloendothelial System. M. S. Thesis. Kansas State Teachers College of Emporia, Kansas.

Panzetta, P.B. Pirofsky, and D. Rigas. 1973. Mechanism of the Mitogenic Action of the Phytohemagglutinin. I. Induction of Tolerance and Lymphocyte Transformation. J. Reticuloendothel. Soc., 13:298.

Pearsall, N.N., R.S. Weiser. 1970. The Macrophage. Lea and Febiger, Philadelphia. p. 57.

Perkins, E.H., P. Nettesheim, T. Morita, and H.E. Walburg. 1967. The Engulfment Potential of Peritoneal Phagocytes of Conventional and Germ-free Mice. Adv. Exp. Med. Biol., 1:175.

Poste, G. and P. Reeve. 1972. Agglutination of Normal Cells by Plant Lectins following Infection with Nononcogenic Viruses. Nature New Biol., 237:113.

Rabinovitch, M. 1967. The Dissociation of the Attachment and Ingestion Phases of Phagocytosis by Macrophages. Exp. Cell Res., 46:19.

_____. 1968. Effect of Antiserum on the Attachment of Modified Erythrocytes to Normal or to Trypsinized Macrophages. Proc. Soc. Exp. Biol. Med., 127:351.

Robbins, J.H. 1964. Tissue Culture Studies of the Human Lymphocyte. Science., 146:1648.

Romeo, D., G. Zabucchi, and F. Rossi. 1973. Reversible Metabolic Stimulation of Polymorphonuclear Leukocytes and Macrophages by Concanavalin A. Nature New Biol., 243:111.

Rott, R., H. Klenk, H. Becht, and Hans-Dieter. 1972. On the Structure of the Influenza Virus Envelope. Virology, 47:579.

Sharon, N. and H. Lis. 1972. Lectins: Cell-Agglutinating and Sugar-Specific Proteins. Science., 177:949.

Singer, S.J., and G.L. Nicolson. The Fluid Mosaic Model of the Structure of Cell Membranes. Science., 175:720.

Sumner, J.B., and S.F. Howell. 1936. The Identification of the Hemagglutinin of the Jack Bean with Concanavalin A. J. of Bact., 32:227.

Van Oss, C.J. and C.F. Gillman. 1972. Phagocytosis as a Surface Phenomenon. I. Contact Angles and Phagocytosis of Non-Opsonized Bacteria. J. Reticuloendothel. Soc., 12:283.