CLEARANCE OF SALMONELLA TYPHIMURIUM AND CARBON BY THE RETICULOENDOTHELIAL SYSTEM IN NORMAL AND SILICA-TREATED MICE

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RICHARD LEE FRIEDMAN

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ABSTRACT

CLEARANCE OF *SALMONELLA TYPHIMURIUM* AND CARBON BY THE RETICULOENDOTHELIAL SYSTEM IN NORMAL AND SILICA-TREATED MICE

By

Richard Lee Friedman

Dorënturp crystalline silica was used as an experimental tool to study bacterial trapping in the isolated perfused mouse liver. Scanning electron micrographs (SEM) of livers from silica-treated mice revealed destruction of Kupffer cells but no other deleterious effects on the liver. Silica treatment decreased bacterial trapping of 10^{10} *Salmonella typhimurium* by the perfused liver from 63.5% in normal mice to 31.3% in silica-treated mice. While silica treatment significantly decreased bacterial trapping by the perfused liver, SEM showed that sinusoidal trapping of *S. typhimurium* still occurred.

In vivo bacterial clearance of *S. typhimurium* from the blood was also depressed with the phagocytic index being decreased from 0.092 in normal controls to 0.058 in silica-treated animals. After intravenous injection of *S. typhimurium* into normal mice, the majority of the

bacteria remaining viable in the host were recovered in the liver, while in silica-treated mice most were recovered in the carcass. Silica treatment enhanced susceptibility to *S. typhimurium* infection in mice over 100-fold. These experiments are the first demonstration of enhancement of gram negative infection by silica. The results obtained from *in vivo* experiments were consistent with the data from the isolated perfused liver and suggested that this *in vitro* technique is an accurate indicator of the state of bacterial clearance in the whole animal.

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By

Richard Lee Friedman

A THESIS

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DEDICATION

To my wife, Ellen Kay Friedman, who has suffered twice
as much through this ordeal and who has put up with
me during these times
To my parents, Sherman and Dorthy Friedman
To my sister, Cynthia Friedman
To my research advisor, Robert J. Moon

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INTRODUCTION

Crystalline silica is specifically toxic for macrophages (1,3,4,19,44,54). Despite this well established observation, relatively little work has been done on the ability of crystalline silica to enhance infections. Silica, *in vitro*, potentiates growth of *Mycobacterium tuberculosis* in macrophage cultures (2) and increases susceptibility to tuberculosis infection *in vivo* (15,86, 87). Crystalline silica also enhances susceptibility to viral (48,95-97) and parasitic infections (46), presumably by suppression of RES activity. To date, data on the effect of silica on enhancement of gram negative infections does not exist.

Three different forms of crystalline silica were initially tested to determine what dose and treatment period was most effective in suppressing carbon clearance from the blood. Carbon clearance is a common method used to study the functional state of the RES (9). Dorënturp silica (10 mg over 3 days) was the most toxic and was used in subsequent experiments.

The primary objectives of this study were to determine whether silica treatment decreases the trapping ability of the perfused liver and whether such treatment causes decreased clearance and/or enhanced susceptibility to gram negative infection *in vivo*. The effects of silica on the perfused liver were studied using colloidal carbon clearance, bacterial trapping and by scanning electron microscopy of liver Kupffer cells. To determine if data from the perfused liver was an accurate indicator of the state of bacterial clearance from the blood *in vivo*, the effects of silica treatment on *in vivo* clearance of colloidal carbon and *Salmonella typhimurium* were also performed. Additionally the effect of silica on the mortality to *Salmonella* infection was examined.

LITERATURE REVIEW

The reticuloendothelial system (RES) is made up of fixed phagocytic cells which include liver Kupffer cells, splenic macrophages, microglial cells of the brain, pulmonary alveolar macrophages and lymph node macrophages. Free or wandering macrophages in the blood are also included in this system. The polymorphonuclear leukocytes (PMN) are not included as part of the RES. One of the major roles played by the RES involves protecting the host from infection by clearing and killing organisms that enter the blood (36,75). Other physiological functions carried out by the RES include hemoglobin uptake and breakdown (30), protein transport (37), uptake and metabolism of steroids and other lipids (6,8,22,61), uptake and degradation of red blood cells (6), phagocytic clearance of autologous tissue debris (88), endotoxin uptake and detoxification (27), phagocytosis of foreign colloidal and particulate material from the blood (9,81), antigen processing for lymphocytes (28) and destruction and immunity to tumor cells (7,24,43).

With respect to the role of the RES in host defense, Rogers reviewed host mechanisms involved in clearing bacteria from the blood stream (75). When large numbers of viable bacteria were injected into the blood, three clearance phases could be distinguished. Ninety to 99.9% of the bacteria were cleared in the first 10 min to 5 h, the rate depending on the bacteria under study. This first phase was followed by a period when microorganisms persisted in the circulation due to slower rates of removal. This second phase lasted for a few hours to several days. During the final phase, bacterial numbers in the blood rose and either disappeared completely or increased to high levels until death occurred.

During the early clearance phase, Rogers stated that the fixed phagocytic cells of the RES sequestered the majority of the bacteria. The cells most active in this process were the liver Kupffer cells and the splenic macrophages. Other RES cells may also have been involved to a lesser extent. The liver cleared the largest numbers of bacteria when clearance was rapid. Sixty to 95% of bacteria cleared in the first 10 min to 5 h were sequestered in these two organs.

The degree to which different bacteria were trapped by the liver and spleen varied from microbe to microbe and was not due to the animal system being used. Staphylococci were cleared quickly by the RES of dog, rabbit and rat while *E. coli* was sequestered less efficiently in these animals. Without antibody containing serum, pneumococci were minimally removed by the splanchnic tissue. The ability of the RES to clear different organisms from the circulation determined the rate at which bacteria were removed during early periods after intravenous injection.

Factors other than the RES were also involved in maintaining sterility of the peripheral blood, particularly the PMN. Intravenous injection of pneumococci or staphylococci caused a profound leukopenia with leukocytes reappearing in the circulation 10 to 20 min later. Stained smears of blood at this time showed bacteria in the cytoplasm of the PMN. It seems that these bacteria caused the leukocytes to stick to endothelial surfaces in capillary beds. White blood cells lodged in lung capillaries were very active in clearing the cocci from the blood. Gram negative organisms, such as *E. coli*, caused prolonged leukopenia of 4 to 6 h. Here it seems more leukocyte damage was caused and that cells that appeared in the following leukocytosis were new PMN.

With high RES sequestering, the PMN probably play a small role in clearance. Polymorphonuclear neutrophils play a major role when the RES is unable to contain certain bacteria. The circulating PMN are a major defense against localized or fixed infections while the RES protects against circulating organisms.

Howard (36) stated that Wyssokowitsch, in 1886, demonstrated that bacteria and fungal spores injected I.V. into rabbits were cleared by specific endothelial cells in the liver and spleen. Bull (16), in 1915, studied the fate of typhoid bacilli (*Salmonella typhi*) after intravenous injection into rabbits. He found that after 15 min the number of bacteria per milliliter of blood dropped from 10^7 to 40 bacteria/ml. Bull looked at the distribution of *S. typhi* in different tissues 21 min after injection and found bacteria mainly in spleen, liver and lung. The numbers of bacteria were much lower than were initially in the blood at 1 min. He stated that the bacilli accumulated mainly in the liver and spleen and were taken up by assembled PMN (actually RES cells) and destroyed.

Manwaring and Coe (52) ran perfusion experiments on different organs of the rabbit to determine which organs were involved in clearing bacteria from the blood. Pneumococci were suspended in Ringer solution with or without immune serum (1%). The perfused liver, in the presence of immune serum, completely cleared the perfusion media of bacteria. Without immune serum only about 25% of the pneumococci were cleared. Intestine, kidney, and hindquarters were also perfused and few bacteria were cleared. The addition of 1% immune serum did not enhance retention of the bacteria by these organs as it did in the liver. Manwaring and Coe called the serum component that causes the pneumococci to adhere to the liver capillary endothelium, endothelial opsonin.

The "laws" of microbial tissue affinity were further studied by Manwaring and Fritschen (53) using other types of microorganisms in perfusion experiments on dogs. Spleen perfused with serum cleared 60% of the *Staphylococcus aureus* in the perfusate while the liver cleared 80%. Other bacteria tested had different hepatic affinities; *E. coli* 40%, *Bacillus biseptius* 10%, *Bacillus anthracis* 25% and *Bacillus lactis aerogenes* only 4%. When immune serum was added to the perfusion, 60% of the *Bacillus lactis aerogenes* was cleared by the liver. Heating immune serum to 60° for 30 min reduced hepatic affinity for the above microbe from 60% to 15%. This study showed that bacteria were mainly cleared by the liver and spleen and that the affinity of bacteria for these organs was increased by serum factors.

Howard and Wardlaw (21) and Wardlaw and Howard (89), using the rat liver perfusion technique, studied how different perfusion fluids affected phagocytosis of bacteria by liver Kupffer cells. When *E. coli* was suspended in Ringer-Locke solution, 11% were taken up by the liver. Forty-one percent were cleared when serum was added.

An attempt was made to delineate what factors were involved in the ability of serum to enhance phagocytosis in the perfused liver. Heating normal human serum at 56°C for 30 min depressed its opsonic activity for *E. coli* but not completely. Serum absorbed with homologous but not heterologous *E. coli* depressed the opsonic activity of the serum. Zymosan absorbed serum also decreased opsonic activity. By both absorbing with homologous bacteria and heating serum to 56°C for 30 min, opsonic activity was almost completely abolished. Only 3% of bacteria perfused in this treated serum were cleared while in untreated serum 41% were cleared by the liver.

Experiments done on gram positive bacteria (89), staphylococci and streptococci showed that they were sequestered in the liver in the absence of serum (85% cleared). With the addition of serum to the perfusate liver clearance diminished to between 67 and 54%, respectively. By these studies Howard and Wardlaw showed that a heat-labile factor (complement) and a heat-stable factor (antibody) in serum enhanced phagocytosis by Kupffer cells in the perfused rat liver. Also they suggested that properdin may play a role.

Using the isolated perfused rat liver technique, Bonventre and Oxman studied the phagocytosis and intracellular survival of *Staphylococcus aureus* and *Salmonella enteritidis* (14). Four different experimental conditions were used: normal rat liver and normal rabbit serum, normal livers with immune serum, immune livers (livers from animals immunized with heat killed *S. aureus* or *S. enteritidis*) with normal serum and immune livers and serum. In the case of *S. aureus*, the immunological state of the liver or serum had no effect on phagocytosis or killing. Clearance and killing were essentially the same under all experimental conditions. *Salmonella enteritidis* was found to be sensitive to both immune serum and immune liver. The presence of immune serum enhanced clearance and intracellular killing in normal and immune livers. The rate of clearance and killing was the greatest when both immune liver and immune serum were used. In this case only 6% of the perfused *Salmonella* survived. In normal serum and liver experiments bacterial levels increased 113% during the 2 h perfusion.

Jeunet et al. (40,41) did liver perfusion experiments using ^{125}I-labeled heat-killed *Brucella melitensis*, *Salmonella typhosa* and *Brucella abortus*. *Salmonella typhosa* and *B. melitensis* were perfused with either non-immune rat blood plus Tyrodes solution, immune rat blood plus Tyrodes or just Tyrodes solution. In all cases clearance of the bacteria was similar. No clearance of *B. abortus* occurred unless specific agglutinating antibody was present in the perfusate. *In vivo* experiments showed that *S. typhosa* and *B. abortus* were both efficiently cleared from the circulation after I.V. injection. *Salmonella typhosa* was found mainly in the liver. The distribution of *Brucella abortus* was vastly different, the majority being cleared by the spleen.

Recent studies have shown that the RES was also involved in clearing fungi from the circulation. Baine et al. (5) found that *Candida albicans*, intravenously injected into rabbits, was cleared quickly from the blood stream. Distribution of the fungi varied depending on the portal of entry. If *Candida* were injected via the peripheral vein, most organisms were trapped in the lungs. Injection of the mesenteric vein found clearance predominantly by the liver. Clearance of *C. albicans* in isolated rabbit liver was performed. Ninety percent of the yeasts were sequestered when perfused in buffer. Addition of 5% normal rabbit serum increased clearance to 98%. In their work Baine et al. made no mention of any killing of the *C. albicans*. Sawyer (77), doing rat liver perfusion and whole animal studies, likewise found no killing of *C. albicans*.

Aspergillus fumigatus spores (10) were injected I.V. into normal rats to study experimental aspergillosis by Turner et al. (84). This treatment resulted in a high incidence of lethal intracranial aspergillosis within 48 to 72 h. *Aspergillus fumigatus* spores were found to be rapidly cleared and destroyed mainly by the lung, liver and spleen. Even with this high rate of clearance and destruction, spores were still able to get into cerebral tissue and cause lethal intracranial aspergillosis.

More recently, Moon et al. (57) have done a study to more clearly define what occurs in the perfused liver. They showed that clearance of *S. typhimurium* by the perfused mouse and rat liver was not synonymous with bacterial phagocytosis and killing by Kupffer cells.

They found that no killing of *S. typhimurium* occurred in perfusion of mouse and rat liver when the perfusion media contained only M-199. Greater than 70% of the *Salmonella* perfused in the livers were cleared in one pass when no blood was in the perfusate. By use of phase and electron microscopy, bacteria in mouse and rat livers were found to be trapped in the liver sinusoids and at times were stacked in the vessels giving a "log-jam" appearance. When whole blood was added to the bacteria and the perfusate in the rat liver perfusion, over 50% of the *Salmonella* were killed. Blood alone caused no killing of the bacteria. These experiments showed that bacterial clearance in liver perfusion did not reflect phagocytosis but actually trapping of *Salmonella* in the liver sinusoids. In order for phagocytosis and destruction of bacteria to occur, whole blood had to be present.

The ability of the RES to clear bacteria or particulate matter from the blood depends on two factors: the state of the RES cells and the presence or absence of humoral factors (antibody, complement, properdin and undefined opsonins). Early studies on the kinetics of RES phagocytosis or clearance were done with many types of inert colloidal suspensions. Such colloids used were: colloidal gold, saccharated iron oxide, heat aggregated serum proteins, lipid emulsion and most commonly colloidal carbon. Biozzi et al. (9) determined the kinetic laws governing the phagocytosis of colloidal carbon and found them to be true for all other test particles as well. By histological examination, 90% of the colloidal carbon injected intravenously into rats accumulated in the liver Kupffer cells and splenic macrophages. Phagocytic activity of these cells could therefore be measured by the clearance of carbon from the blood. The early rate of clearance is independent of the initial carbon concentration. The rate of phagocytosis is directly proportional to the blood concentration and inversely proportional to the amount of carbon already phagocytized. Thus, particles are phagocytized according to an experimental function of their blood concentration in relation to time. Phagocytic index (K) is the measure of the rate of phagocytosis of particles from the blood. The phagocytic index is inversely proportional to the dose of particles injected (D) or KD = cte; where c is blood concentration of colloid and t is time. To determine the phagocytic function of the RES, one must use a dose of carbon greater than the critical dose: critical dose = KD/K_{max} Under these conditions (using saturating dose) the phagocytic index can be determined.

Biozzi et al. (9) and Stiffel et al. (81) stated that the decrease in phagocytic function resulting from the uptake of a given dose of colloid after its blood clearance caused a saturation or blockade of the RES cells. In effect the reduction in phagocytosis was due to physical overloading of macrophages by colloid. They stated that colloidal clearance was not influenced by humoral factors.

Much controversy has surrounded the role of serum opsonins in colloid clearance. Jenkin and Rowley (38) demonstrated that clearance of bacteria and colloidal carbon from the blood of mice requires serum factors in order to be phagocytized by RES cells. Mice were injected with a very high dose of carbon to produce RES blockade. Then the rate of clearance of either serum opsonized or unopsonized carbon was studied in these mice. Unopsonized carbon clearance was greatly impaired but the opsonized carbon was cleared at a rate similar to that found in untreated mice. From this experiment Jenkin and Rowley felt RES blockade was due to exhaustion of serum opsonins for colloid carbon.

Jeunet and Good (39) found that RES blockade to colloidal gold, in the perfused rat liver, could be completely reversed to normal by addition of fresh rat plasma to the perfusion. Filkens et al. (25,26) obtained similar results when colloidal carbon clearance in perfused rat liver was studied. They found that a plasma factor was required for carbon clearance to occur normally.

Studies done by Murray (59,60) showed that RES blockade was also due to depletion of plasma factors.

He found that blockade to a tracer dose of colloid occurred only when the surface properties of both blockading and tracer particles were identical. Colloidal carbon is kept in suspension by use of fish glue, a type of gelatin, and by regular gelatin. In effect, then, it can be defined as a gelatin-coated particle. Thus, the blockading effect of carbon is dependent on the surface properties of the particle, i.e., gelatin, and not on the nature of the particle itself. Murray stated that the decrease in clearance in RES blockade was due to gelatin decreasing the level of opsonins in the plasma. He found that the degree and duration of RES blockade were directly related to the circulating levels of gelatin agglutinins. Experiments by Normann et al. (65) seem to confirm Murray's work. Normann et al. found that carbon clearance was dependent on the gelatin concentration in the carbon suspension. As the ratio of carbon concentration to gelatin became less (more gelatin present), the phagocytic index decreased in a linear fashion. He stated gelatin mediates its effect by direct action on the carbon particle surface. It either alters its relationship with blood constituents or affinity of RES cells for carbon. Gelatin injected during the course of carbon clearance did not alter the carbon removal rate, thus supporting the above conclusion.

While many authors have shown that colloidal RES blockade was due to depletion of opsonic factors, other workers supported the concept that blockade was due to saturation of the RES cells. Biozzi et al. (12) did experiments similar to those of Jenkin and Rowley (38). They found that incubation of carbon with normal sera from different animals had no effect on the rate of carbon clearance in animals previously treated with blockading levels of carbon. Lireman et al. (50) were also unable to find serum factor depletion involved in RES blockade in the rabbit.

While there is still disagreement on the role of serum factor involvement in colloidal particle clearance, their role in bacterial clearance is well established. Early liver perfusion experiments showed that for most bacteria, RES clearance was vastly enhanced by the presence of normal or immune serum (35,38,40,41,52,53,89). Moon et al. demonstrated that without the presence of whole blood no killing of *Salmonella typhimurium* occurred in the perfused rat liver (57). Others have also shown the role of serum factors in RES bacterial clearance (11,12,58,76).

To further understand the role of the RES in resistance to infection, experimental depression of the RES has been employed. Techniques, besides inert colloidal suspensions, used to suppress RES functions have been: x-irradiation (6,31), anti-Kupffer cell serum (70), anti-lymphocyte serum (32,71), cyclophosphamide (80), cortisone (64,83,90), throtrast (91,92), ethyl palmitate (82), and methyl palmitate (13,23). It is not known precisely how many of these agents cause their depression of the RES. With these methods it is doubtful that the RES cells are the only cells being affected by the treatment. Thus, a more specific depressant of the RES is required to study its role in host resistance to infection. One such compound, crystalline silica, has been found to be toxic only for macrophage (1,44).

Crystalline silica is just one of many different forms of silica (silicon dioxide) which exists on the earth. Other types are amorphous or vitreous silica, diatomaceous earth quartz, sandstone, flint and asbestos. All forms of silica are composed of silicon and oxygen atoms arranged in a tetrahedron structure, with the silicon atom at the center and the oxygen atoms at the four corners. Each oxygen atom is shared by an adjacent silicon atom. With this tetrahedral arrangement, silica has a three-dimensional lattice macromolecular structure. Most silicas contain SiOH groups (silanol groups) on the surface. These phenolic hydroxyl groups can form hydrogen bonds with water, producing an outer layer of free or adsorbed water on its surface. The silanol groups can also react with other hydrogen acceptors (see below). The spatial arrangement of the tetrahedra can vary and this accounts for the various types of silica. Vitreous silica has an irregular pattern; crystalline silicas, such as DQ12 quartz and tridymite, have a characteristic regular three-dimensional tetrahedral structure (3,15).

Interest in the toxicity of silica particles increased when it was realized that silica dust could cause fibrotic lesions, similar to tubercule granuloma, when inhaled into the lung. The disease, silicosis, has been reported common among miners and is described as the progressive development of fibrous tissue, in a nodular or diffuse form, in response to a dust. Many theories of how silica causes the pathological lesions of silicosis have developed over the years.

One early hypothesis to explain silica toxicity was the mechanical theory. It was thought that the particles caused damage by the cutting and laceration of tissue with which it came in contact. Microscopically, silica particles have very sharp edges and points. Gardner (29) disproved this theory by showing that silicon carbide dust, which also has sharp edges like silica, does not produce silicosis when inhaled by test animals.

A second explanation of silica toxicity was the solubility or chemical toxicity theory. According to the theory, silica gradually dissolved as it was bathed in body fluids, liberating silicic acid which acted as a tissue toxin and caused fibrosis (15,47). Gye and Purdy (33) were able to produce fibrotic lesions in the liver of mice after injection of silicic acid or amorphous silica. Kettle (45), by implanting silica in a sac subcutaneously in rabbits, obtained local fibrosis. King's in-depth study on the solubility theory of silicosis shed much doubt on this explanation (47). King compared the solubility of different types of silica with their pathogenicity. He found that pathogenicity did not correlate at all with solubility of the test particles. Sandstone was found to have low solubility and yet was very toxic to animals. Twenty Angstroms silica had high solubility but low pathogenicity. Quartz had high solubility and high pathogenicity. King, who initially was a proponent of the solubility theory, felt from these experiments that a better explanation must be sought to explain the fibrogenic action of silica.

The experimental work of Curran and Rowsell (21) was also evidence contrary to the solubility theory. Diffusion chambers made from millipore filters containing silica were placed subcutaneously into rats. After several months, the implanted area was checked and no fibrogenic reactions were found. Scheel et al. (78) found that silicic acid and its salts were non-toxic and that silicic acid in body fluids were rapidly eliminated by the kidney.

The autoimmune theory of silicosis was advanced by Vigliani and Pernis (85). They suggested that the basic lesion in silicosis was started by silica uptake by macrophage where it damages the cell. These damaged cells would then liberate their contained antigens and stimulate an antibody response. The resultant antigen-antibody reaction would precipitate at the site of antigen release, producing the hyaline substance in the silicotic nodule. Composition of the acellular part of human silicotic nodules was studied. It was found that 40% of the hyaline substance was collagen and 60% gammaglobulin.

Silica has been shown to have an adjuvant effect on antibody production (69,85). Pernis et al. (69) found that pretreatment of rats or rabbits with tridymite I.V., before immunization with ovalbumin or horse serum, greatly enhanced antibody production to these antigens. They stated that the adjuvant effect of silica may cause proliferation of the RES and that this might play a role in silicosis. Vigliani and Pernis (85) reasoned that lipopolysaccharide liberated from the cell wall of destroyed macrophages might act as an autoantigen and initiate the whole pathological process. To date there has been no evidence to back this assumption. Scheel et al. (79) showed that quartz could absorb proteins and denature them. They proposed that this may result in the production of a foreign protein reaction in tissue.

Razumov et al. (72) proposed a theory of silicosis based on the piezoelectric effect. Quartz crystals have no center of symmetry and thus are piezoelectric and manifest optical rotation. He states:

> Individual particles of quartz crystals or groups of particles, when introduced into the animal tissues, evidently react intimately with them, transforming the electrical energy of the fields arising in the course of metabolic processes in living tissue into mechanical energy. On the other hand, the mechanical energy of the organ when transmitted to the implanted crystalline particle, may thereby be transformed into electrical energy. In this way, an alternating electric field may develop around the crystals, which in turn disturbs the normal course of tissue metabolism and energy exchange.

The most recent theory of silicosis is that of Allison (1,3,4) and Page et al. (67). This theory is that silica is specifically cytotoxic for macrophage and after ingestion, the particles interact with the membranes of secondary lysosomes and make them permeable. Lysosomal enzymes leak into the cytoplasm causing cell destruction. With the destruction of the macrophage, lysosomal enzymes and a fibrogenic substance are released causing the pathological lesion of silicosis.

Crystalline silica has been shown to be a specific toxin for macrophage (1,19,44,54). Marks et al. (54) studied the *in vitro* effects of different dusts on guinea pig peritoneal macrophage. Crystalline silica was found to reduce dehydrogenase activity in half by 3 days at a dose of 2-3 micrograms per 3×10^6 macrophages. Other dusts like coal or kaolin had no toxic effect until 800 micrograms per 3×10^6 cells were used.

Experiments of Kessel et al. (44) studied the effects of tridymite, a crystalline silica, tridymite coated with amorphous silica, vitreous silica, hematite and carbon on viability of guinea pig peritoneal macrophage *in vitro*. Viability was determined by the rate of lactic acid production. The number of macrophage and the amount and size of the particles used in each experiment were kept constant. Tridymite was found to be the most toxic compound and completely inhibited lactic acid production after 7 h incubation. Hematite, carbon and vitreous silica treated macrophages had normal levels of lactic acid production during the same time period. Coating tridymite with amorphous silica vastly decreased tridymite macrophage cytotoxicity. Lactic acid production was near normal in these treated cells. Kessel et al. showed by this experiment that the characteristic cytotoxicity of silica was mainly involved with properties of its surface. Tridymite was also found to be toxic for macrophage of rabbit, rat, mouse and man. The other test particles had no toxic effect.

Other phagocytic cell types were studied for crystalline silica cytotoxicity. Polymorphonuclear leukocytes from guinea pigs and rabbits were unaffected by tridymite after its phagocytosis. No toxicity was shown for monkey kidney cells, KB cells, HeLa cells, mouse fibroblasts, guinea pig fibroblasts or for chick embryo skin, liver, muscle, or heart cells. All these cell types actively phagocytose crystalline silica with no decrease in lactic acid production. Kessel and co-workers showed that crystalline silica with an appropriate surface was specifically cytotoxic to only one cell type, the macrophage.

Effects of silica, diamond dust and carrageenan on mouse peritoneal macrophages were studied by Allison et al. (1), utilizing phase-contrast cine-micrography, electron microscopy, histochemical techniques for lysosomal enzymes and measurement of the release of lysosomal enzymes into the culture medium. They found that the initial phagocytosis of the silica and diamond dust was the same and that phagosomes developed normally in both cases. Macrophage that took up diamond dust remained viable for at least 30 h, while those phagocytizing silica were dead by 15 h. Histochemical studies were done on cells taking up either silica or diamond dust. Acid phosphatase was used as a lysosomal enzyme marker. In cells that engulfed diamond dust, phosphatase activity was confined within the phagosomes and only low levels of lysosomal enzymes were released into the culture medium. Cells that phagocytosed silica had diffuse cytoplasmic phosphatase staining and high levels of lysosomal enzymes were released into the culture medium. Cells that phagocytosed silica had diffuse cytoplasmic phosphatase staining and high levels of lysosomal enzymes in the culture medium. Electron micrographs showed that silica, initially after phagocytosis, was within phagosomes. By 14 h silica particles were seen free in the cytoplasm. Other morphological changes were swelling and rounding up of mitochondria and even complete disintegration of cytoplasmic structure.

Allison et al. suggested that crystalline silica was cytotoxic for macrophages because it was taken up readily by the cell and silica could then react with the membranes surrounding the secondary lysosomes, making them permeable. Lysosomal enzymes and silica could then get into the cytoplasm, causing death of the cell, and were released into the culture media. Silica released from dead macrophages was just as cytotoxic as the original silica preparation. *In vivo* these released particles could be taken up by other macrophages causing further killing. This cycle would continue unless the silica was somehow sequestered.

Nadler et al. (62) confirmed this phenomenon by the diaminobenzidine technique with E.M. They found that macrophage ingesting silica do release their lysosomal enzymes into the cytoplasm of the cell. Scanning electron microscopy by Hope and Friend (34) on macrophages cultured with crytalline silica also confirmed Allison's results.

Allison et al. (1) and Nash et al. (65) have also presented evidence that crystalline silica in suspension, due to its many phenolic hydroxyl groups on its surface, could act as a powerful hydrogen bonding agent. These phenolic hydroxyl groups could react with secondary amide groups of proteins causing denaturation (79). The interaction with phospholipids was even stronger and by this action could cause lysosomal membranes to become permeable. The rigid structure of the silica crystal was found to be important because silicic acid and other non-crystalline forms of silica were much less toxic to macrophage. As Allison stated:

> It seems that the rigid structure of the quartz, with many hydrogen bonding groups arranged in a regular and immovable order on its surface, must be important in damaging cells. Presumably, the formation of multiple bonds distorts the membrane structure thereby leading to the breakdown of the membrane.

Support for the concept that H-bonding is important in silica cytotoxicity also comes from experiments using poly-2-vinylpyridine N-oxide (PVPNO) (1,52,65,73). Allison et al. (1) found that this polymer, when taken up by macrophage into their secondary lysosomes, protects the cell from silica that was later phagocytized. The cytotoxicity of crystalline silica could also be inhibited when PVPNO was first mixed directly with the particles to coat them. Nash et al. (65) pointed out that PVPNO has oxygen atoms which can hydrogen bond with the silanol groups on the surface of silica particles. PVPNO prevented silica from interacting with the lysosomal membrane, and in this manner protected the cell.

Silica has been used to show the role of macrophage in allograft rejection (51,68,72). Pearsall and Weiser (68) showed that intraperitoneal (I.P.) injection of silica in mice prolonged skin allograft survival time if given before or after grafting. Silica had no effect when given at the time of grafting. Lotzova and Cudkowicz (26) in experiments with allogeneic bone marrow grafts, and Rios et al. (73) with allogeneic skin grafts, found that silica prevented rejection of the allograft. PVPNO administration to the grafted animals inhibited the immunosuppressive effect of silica and allowed graft rejection to occur. Keller (42), by the use of silica, showed that the macrophage plays a role in restricting tumor cell proliferation *in vitro*. Zarling et al. (94) found the same to be true in *in vivo* experiments.

Only limited studies are available on enhanced susceptibility to infections in silicotic animals or man. Human patients with silicosis have a higher than normal incidence of tuberculosis (86). Vorwald et al. (87) found that when BCG was administered to silicotic guinea pigs a progressive tuberculosis developed, while in normal animals BCG injection did not cause the disease. The effect of silica on the growth of *Mycobacterium tuberculosis* in macrophage cultures was studied by Allison et al. (2). Their experiments showed that the growth of *M. tuberculosis* H37Rv in macrophage culture was increased by the addition of sublethal doses of silica.

Silica-treated macrophages had earlier multiplication of the bacteria and cells died and released the organisms sooner than cells, either infected without silica or treated with just silica. If these two factors--faster multiplication and earlier release of bacteria from macrophage--occur *in vivo* as well, it could greatly enhance the spread of tuberculosis in patients with silicosis.

The role of the RES in viral and parasitic diseases has been studied in experimental animals treated with silica to depress RES functions (46,48,95-97). Zisman et al. (95-97) treated mice I.P. or I.V. with silica to determine the role of the RES in viral infections. When normal mice were injected I.P. with herpes simplex virus, only 18% of the mice died (95). Before death the animals developed focal meningitis but no other organs showed histological changes. If mice were injected I.P. with crystalline silica, 2 h before the virus, mortality was 73%. In the silica-treated mice high viral titers developed in the liver with severe hepatic necrosis. Silica treatment alone caused no deaths.

Adult mice were normally not susceptible to yellow fever virus infection by the intravenous or intraperitoneal routes (80-100% resistant). When mice were first treated with silica I.V. and then injected 2 h later with virus, over 95% of the mice died of the yellow fever viral infection (96). Similar experiments were done with coxsackie B-3 virus (97). Mice treated with silica had a total mortality of 70% to viral inoculation, while only 25% of the normal mice succumbed to the infection.

Kierszenbaum et al. (46), in trying to determine the role of the RES in resistance to *Trypanosoma cruzi*, treated mice intravenously with crystalline silica. Silica was found to decrease resistance to infection with virulent blood forms of the parasite. Animals given silica had both a higher mortality rate and higher levels of parasitemia than controls.

Recently Levy and Wheelock studied the effects of silica on immune and non-immune functions of mice (49). Carbon clearance in intravenously injected silica-treated mice was significantly depressed 2 h after injection and this depression persisted for 3 days. These data correlate well with the enhanced susceptibility of silica-treated animals to viral, parasitic and *M. tuberculosis* infections. Silica had no effect on the induction of interferon by chlorite-oxidized-oxyamylose-statolon or by Newcastle disease virus. Intravenous silica did not greatly alter the number or composition of peritoneal exudate cells (PEC). Macrophage isolated and cultured from PEC were found to have a 40% reduction in their phagocytic activity as determined by uptake of opsonized sheep red blood cells (SRBC) in a 30 min period. Silica given 1 to 3 days before I.P. injection of SRBC inhibited the production of splenic anti-SRBC-plaque forming cells.

No inhibitory effect was noted when animals were treated 2 h before or after SRBC immunization.

Silica inhibited the cell-mediated immune response of mice to histoincompatible fibroblasts inoculated I.P., when given 3 days before the fibroblasts. The cytotoxic activity of spleen cells of silica-treated immunized mice was less than half of the activity in normal immunized mice. Concanavalin A (ConA) stimulation of the uptake of ^{3}H-thymidine by spleen cells of silica-treated mice was first stimulated and then depressed. The response may be due to the combined consequences of macrophage depletion, release of lymphocyte stimulatory factors and the action of suppressor lymphocytes. Miller and Zarkower (55,56) reported that silica dust inhalation in mice alters the activity of macrophage and also B and T lymphocytes. They found T cell response to ConA was increased and B lymphocyte response to lipopolysaccharide was depressed. Levy and Wheelock feel that these effects on T and B lymphocytes were not due to the direct action of silica on the lymphocytes but rather due to impairment of macrophage directed or dependent functions.

MATERIALS AND METHODS

Animals

Spartan (HA/ICR) female mice (Haslett, Michigan) weighing 18 to 25 g, were used in all experiments. Animals were kept under standard laboratory conditions. Water and Purina Chow were available *ad libitum.*

Bacteria

Salmonella typhimurium strain SR-11 was used in all investigations. Eighteen to 24 h cultures of the organism, grown in brain heart infusion broth, were centrifuged at 8,000 rpm for 15 min. The bacteria were resuspended in M-199 (Gibco, Grand Island, NY) or sterile saline (depending on the experiment). For quantitative plate counts, an aliquot was removed and appropriate dilutions were made in deionized water to prevent bacterial clumping. Duplicate dilutions were plated on tryptose agar to quantitate the viable organisms. The bacterial concentration used in both liver perfusion and whole animal studies was 1-2 x 10^{10} bacteria/ml and in whole animal bacterial clearance and killing studies 1-2 x 10^{9} bacteria/0.1 ml.

Silica

Three types of crystalline silica were studied for their effects on suppressing RES activity. The most effective for this study was Dorënturp silica, particle size 5 μ or less (DQ12) (74). DQ12 was kindly provided by Dr. Gustavo Cudkowicz, Department of Pathology, State University of New York at Buffalo, NY. Ten milligrams of DQ12 silica was injected I.V. over a 3 day period into mice before they were used for experiments on the fourth day after treatment. Crystalline silica (Spex Industries, Meterchen, NJ, no. 03741) preparation of particle size less than 5 μ was prepared by the Cummings sedimentation technique (20). Another silica, 5 μ min-u-sil silica (Wittaker, Clark and Daniels, South Plainsfield, NJ) was also tested. Injection regimen was the same as for Dorënturp silica except doses of 4 mg to 9 mg were used. The silica was autoclaved in powder form and then suspended in sterile saline. Before injection the silica solution was exposed to ultrasonic vibration in a Bronsonic Ultrasonic cleaner (no. B220, Branson Co., Sketon, CN) to resuspend the silica evenly.

Mouse Liver Perfusion

Before surgery mice were given 200 U of heparin (Upjohn Co., Kalamazoo, MI) followed by 1.5 mg of pentobarbital sodium (Haver Lockhart Laboratories, Shawnee, KN) intravenously. A medial abdominal incision was made and the intestine was moved to expose the portal vein and the inferior vena cava. A silk suture was introduced under the vana cava and above the right renal vein. Cannulas were made from 20-gauge needles which were filed until the beveled end was blunt. The cannulas were autoclaved before using.

The portal vein was held taut with forceps approximately 1 cm from the liver, and a small cut was made in the vein between the forceps and the liver. A cannula filled with sterile M-199 was inserted in the vein and tied in place with the suture. When the cannula was in place, it was attached to Tygon tubing (inner diameter 0.31 inch; outer diameter 0.93 inch) which was connected to a three-way valve (Becton-Dickinson and Co., model no. 3160) via an 18-gauge needle. One port of the valve held a 1 ml syringe containing sterile M-199 and the second port was attached to two 50 ml glass syringes filled with pre-warmed, sterile M-199. Before the perfusion was started, the inferior vena cava was cut below the right kidney, to avoid swelling of the liver once the perfusion started. Perfusion medium then flowed in through the portal vein and out the inferior vena cava.

The thoracic cavity was then excised and a ligature was placed under the superior vena cava above the liver. A small incision was made in the right atrium, and the efferent metal cannula was inserted and tied in place.

A 10-20 cm piece of Tygon tubing (inner diameter 0.31 inch; outer diameter 0.93 inch) was then attached to the cannula.

With the second cannula in place, the suture above the right renal vein was tied, which diverts the flow of M-199 into the efferent cannula. During the experiment the liver was kept moist by bathing it with sterile M-199 and keeping the specimen covered with a petri dish to slow evaporation.

The liver was then perfused with M-199 until the effluent was clear of blood. Flow rate during the first few minutes of the perfusion was carefully adjusted until the equilibrium of the circulation had been established and a constant flow rate was maintained. Effluent was tested for sterility, and any liver effluent having >10 colony-forming units (CFU) of bacteria per milliliter at this point was omitted from the final tabulation of data.

After washing, the 1-ml syringe on the 3-way valve which contained M-199 was replaced with a syringe filled with 1 ml of *S. typhimurium*. The bacteria were slowly and steadily infused and followed immediately by 50 ml of M-199. Effluent was collected from the efferent cannula into a sterile 50 ml graduated cylinder. A 1 ml portion after the completion of the perfusion was obtained. This aliquot was plated and the data showed that <0.001% of the infused bacteria continued to be washed out of the liver. Perfusions lasted 30 min or less and were done at room temperature. Liver oxygenation was not specifically maintained.

The effluent was kept on ice until quantitative tryptose agar pour plates were made. The percent of non-trapped bacteria was calculated by the formula:

$$\frac{\text{number of CFU recovered in effluent}}{\text{number of CFU infused}} \times 100$$

The difference between the percentage of recovery in the effluent and the total infused (100%) suggested the percentage of bacteria trapped by the liver.

The liver was disconnected from the perfusion apparatus and homogenized in 9 ml sterile deionized water in a glass homogenizing tube with a teflon pestle and a TRIR-STIR-R. Appropriate dilutions were made and subsequent quantitative tryptose agar pour plates were used to determine the number of trapped viable bacteria still remaining in the liver. The percentage of viable bacteria remaining in the liver plus the percent recovered in the effluent could then be subtracted from the percentage of bacteria infused (100%) to give the percent killing:

% killing = 100% - (% in homogenate + % in effluent).

<u>Carbon Clearance in the Whole Animal</u>

Normal and silica-treated mice were tested for their ability to clear carbon from their blood by the Biozzi et al. technique with modification (9). A carbon preparation, containing 100 mg carbon/ml, was used (Pelikan Carbon Suspension C11/143/a; Günther Wagner, Hanover, Germany). The carbon was diluted to 50 mg/ml in saline. Five milligrams of carbon was injected intravenously into each mouse. The mice were bled from the retro-orbital plexus at 2 min and 15 min after injection. At each bleeding 0.05 ml of blood was obtained and lysed in 4.0 ml of 0.1% Na_2CO_3. The concentration of carbon was determined photometrically (O.D.) in a Hitachi Perkin Elmer spectrophotometer (model no. Coleman 111) using tungsten light at a wavelength of 650 nm. The phagocytic index K was determined for each mouse by the equation:

$$K = \frac{\log C_1 - \log C_2}{T_2 - T_1}$$

where C_1 and C_2 represent the blood colloid concentration at time 1 (T_1) and time 2 (T_2), respectively. The clearance of carbon from the blood can also be expressed in terms of a biological half-life ($T½$) in the blood:

$$T½ = \frac{0.301}{K}$$

Carbon Clearance in the Perfused Liver

Liver perfusion was set up as described above. The liver was then infused slowly with 1 ml of M-199 containing 5 mg of carbon instead of bacteria. Fifty milliliters of effluent was collected. Concentration of carbon remaining in the effluent was determined by O.D. readings at 650 nm. From the O.D. reading, the number of milligrams of carbon in the effluent was determined graphically. The milligrams of carbon cleared by the liver was calculated by subtracting the milligrams of carbon in the effluent from 5 mg of total carbon infused.

Whole Animal Bacterial Clearance

Mice were injected intravenously with 1.0×10^9 viable *S. typhimurium* in 0.1 ml. At 2, 5, 10 and 15 min after injection, 0.1 ml of blood was obtained from the retro-orbital plexus. The blood sample was diluted in sterile deionized water and quantitative tryptose agar pour plates were made. K was determined by the equation:

$$K = \frac{\log \text{CFU 2 min} - \log \text{CFU 15 min}}{\text{13 min}}$$

$T\frac{1}{2}$ was calculated as previously described.

Whole Animal Bacterial Killing

Mice injected intravenously with 1.0×10^9 *S. typhimurium* were killed either 15 or 30 min later by cervical dislocation. The liver and spleen were removed and homogenized separately with a teflon and glass homogenizer in 9 ml of sterile deionized water. The carcass, excluding the stomach, intestinal tract, skin, paws and tail, was homogenized in 99 ml of deionized water in a Waring blender for 3 min. Quantitative pour plates were prepared from the three homogenates.

White Blood Cell (WBC) and Differential Blood Counts of DQ12 Silica-Treated Mice

The blood picture of DQ12 treated mice was followed before treatment and during the period of silica treatment. White blood cell counts were done by bleeding the mice from a lateral tail vein. The blood was drawn by use of a WBC pipette and then mixed with a 2% acetic acid solution. Counts were made by use of a hemacytometer. Differential counts were done by staining blood smears via the Wright-blood stain method. One hundred white blood cells were counted to determine the percent PMN and monocytes.

Effect of DQ12 Silica on *S. typhimurium* Infection in Mice

Mice were treated with 10 mg or 3 mg of DQ12 silica and divided into three separate groups of 6 mice. One group served as a control and the others were injected I.V. with either 2×10^5 or 2×10^3 *S. typhimurium*. Survival of the treated animals was observed for 2 weeks. No deaths occurred in the silica control groups during this time. The LD_{50} for normal mice was determined using the above doses of bacteria plus doses of 2×10^6 and 2×10^4.

Scanning Electron Microscopy (SEM) of Mouse Liver

Livers were infused with bacteria as previously described. Following the perfusion, 5-10 ml of glutaraldehyde was perfused (2.5% glutaraldehyde in 0.2 M phosphate buffer at pH 7.4), for 10 to 20 min. Fixed livers were excised and cut into small blocks and dehydrated in sequential 15 min steps with 30, 50, 70, 90 and 100% ethanol. The blocks were allowed to stand overnight at 4°C in fresh change of 100% ethanol. The dehydrated blocks were cryofractured by immersing them in liquid nitrogen for 1 min. The tissue was fractured with a precooled single edge razor blade held by locking forceps. The fractured tissue was placed in metal baskets under liquid nitrogen and transferred to the critical point dryer. The tissue was dried in an Omar SPC 900/EX critical point dryer using CO_2 as the carrier gas. The dried specimens were moumted on stubs using double stick Scotch Brand Tape and the stub edge painted with television Tube Koat (G.C. Electronics) to prevent charging. The specimens were coated with gold (200-300 Å) using the EMS-41 Minicoater (Film Vac. Inc., Englewood, NJ) and viewed in an AMR-900 scanning electron microscope. Micrographs were made with Kodak positive/negative (P/N) film.

Statistics

Where appropriate, data were evaluated by the White Rank Order method (93).

RESULTS

Effects of Various Silica Preparations on Carbon Clearance

Carbon clearance has been extensively used to monitor the phagocytic potential of the RES (9). The procedure was used to determine which of three types of crystalline silica being tested depressed RES activity the greatest. Each silica was tested at various doses and for varying time periods. The particle size of all silicas was 5 μ or less. In normal mice the phagocytic index was calculated to be 0.056 and the biological half-life 5.38 min (Table 1). Ten milligrams of Dorënturp silica (DQ12) given I.V. over 3 days significantly decreased the phagocytic index (K) to 0.020 and increased the $T\frac{1}{2}$ to 15.15 min. All other doses and types of silica had no significant effect on carbon clearance. Dorënturp silica (10 mg over 3 days) was used in all succeeding experiments.

Scanning Electron Microscopy (SEM) of Livers from Normal and Silica-Treated Mice

Comparative study of liver SEM from normal and silica-treated mice showed no indication of detrimental effects of silica on the portal veins, hepatic veins, central veins, sinusoids and parenchymal cells of the

Table 1. Effect of different preparations of silica on RES carbon clearance[a]

Type of Silica	Phagocytic Index (K)	Biological Half-Life ($T_{1/2}$) (min)	P
Control	0.056	5.38	---
Dorënturp 10 mg over 3 days	0.020	15.15	0.001
Spex 15 mg over 3 days	0.050	6.02	N.S.
5 μ Min-U-Sil 9 mg over 3 days	0.068	4.43	N.S.

[a]Average value from at least eight separate experimental determinations.

liver. The only damaging effect seen was on liver Kupffer cells. Kupffer cells in silica-treated livers:

1. Were not as spread out as normal cells, many being rounded up (Figure 1B, 1D).

2. Were found engorged with silica and appeared to have part or all of their outer plasma membrane destroyed (Figure 1C-E).

3. Had few if any membrane appendages (pseudopodia) as compared to normal K-cells (Figure 1C-E).

4. Had fewer attachment sites to walls of the sinusoids (Figure 1A-E).

The crystalline-like material found in many of the Kupffer cells looked similar to SEM of Dorënturp silica (Figure 1F). Based on these micrographic comparisons, it

Figure 1. Scanning electron micrographs of Kupffer cells from mouse livers. (A) Kupffer cells from normal controls (x 1,450); (B-E) Liver Kupffer cells from silica-treated mice (x 5,200); (F) SEM of Dorënturp silica (x 6,500).

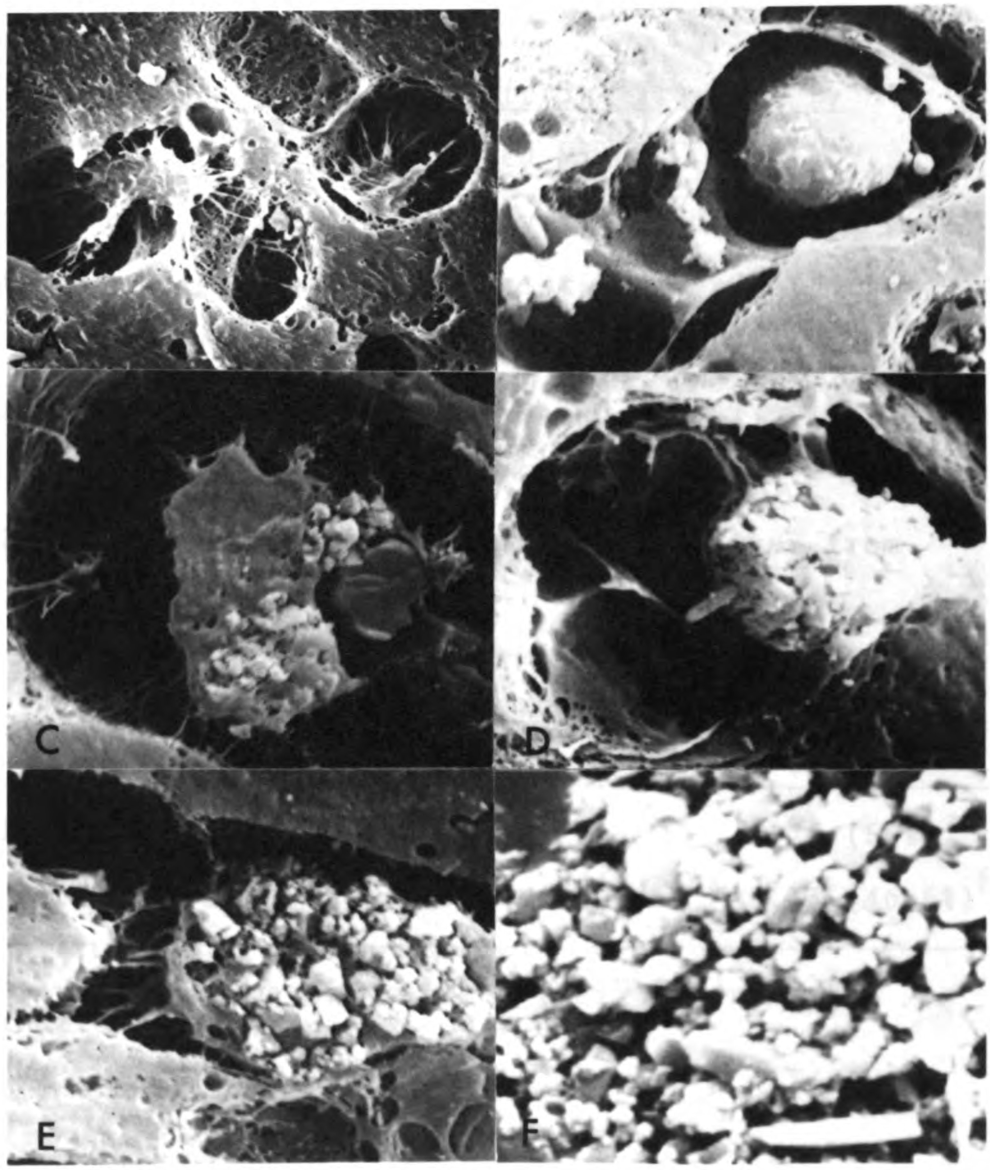

Figure 1

was concluded that the material seen in Kupffer cells was crystalline silica. The SEM work shows that RES cells are destroyed by DQ12 silica.

Carbon Clearance and Bacterial Trapping by Perfused Livers of Normal and Silica-Treated Mice

Normal and silica-treated livers were perfused with 5 mg of colloidal carbon. A statistically significant though not dramatic decrease in carbon clearance was observed in livers from silica-treated mice (Table 2).

Table 2. Carbon clearance in the perfused liver from normal and silica-treated mice[a]

Treatment	Mg carbon in the: Effluent	Liver
Control	2.25 (45%)	2.75 (55%)
Silica treated	2.67 (53.4%)[b]	2.33 (46.6%)[b]

[a]Average value from at least six experimental determinations.

[b]$P < 0.001$.

Thus 55% of the carbon was cleared by normal livers while 46.6% was taken up by silica-treated livers ($P<0.001$). In normal livers perfused with 1-2 x 10^{10} *S. typhimurium*, 63.5% of the bacteria were trapped in the liver and 42.3% of the organisms were recovered in the effluent (Table 3). Silica-treated livers trapped only 31.3% ($P<0.001$), with

Table 3. Clearance of viable *S. typhimurium* by perfused mouse livers from normal and silica-treated animals[a]

Treatment	% in: Effluent	% in: Liver Homogenate	% Total Recovery
Control	42.3	63.5	105.8
Dorënturp silica	65.9 ($P < 0.05$)	31.3 ($P < 0.001$)	97.2 (N.S.)

[a]Average of eight separate experimental determinations.

65.9% appearing in the effluent ($P < 0.05$). Scanning electron micrographs from livers perfused with bacteria show that the liver was still able to trap *S. typhimurium* in sinusoids, even though many Kupffer cells had been destroyed (Figure 2). In both silica-treated and normal livers, approximately 100% of the perfused bacteria were routinely recovered.

Clearance of *S. typhimurium* from the Blood of Normal and Silica-Treated Mice

Figure 3 shows that silica treatment significantly decreased the rate of whole animal bacterial clearance. The phagocytic index for *S. typhimurium* clearance in normal mice was 0.092 with a biological half-life of 3.27 min. The decrease in the clearance of bacteria in DQ12 treated animals was statistically significant at all time

Figure 2. Scanning electron micrographs of silica-treated livers perfused with 1-2 x 10^{10} *S. typhimurium*. (A) Bacteria trapped in sinusoid, note Kupffer cell lysed by silica (S) and rounded up Kupffer cell (K) (x 2,600); (B) Sinusoid bacterial trapping (x 6,500); (C) Bacteria trapped in sinusoid with rounded up Kupffer cell at sinusoidal junction (K) (x 5,800).

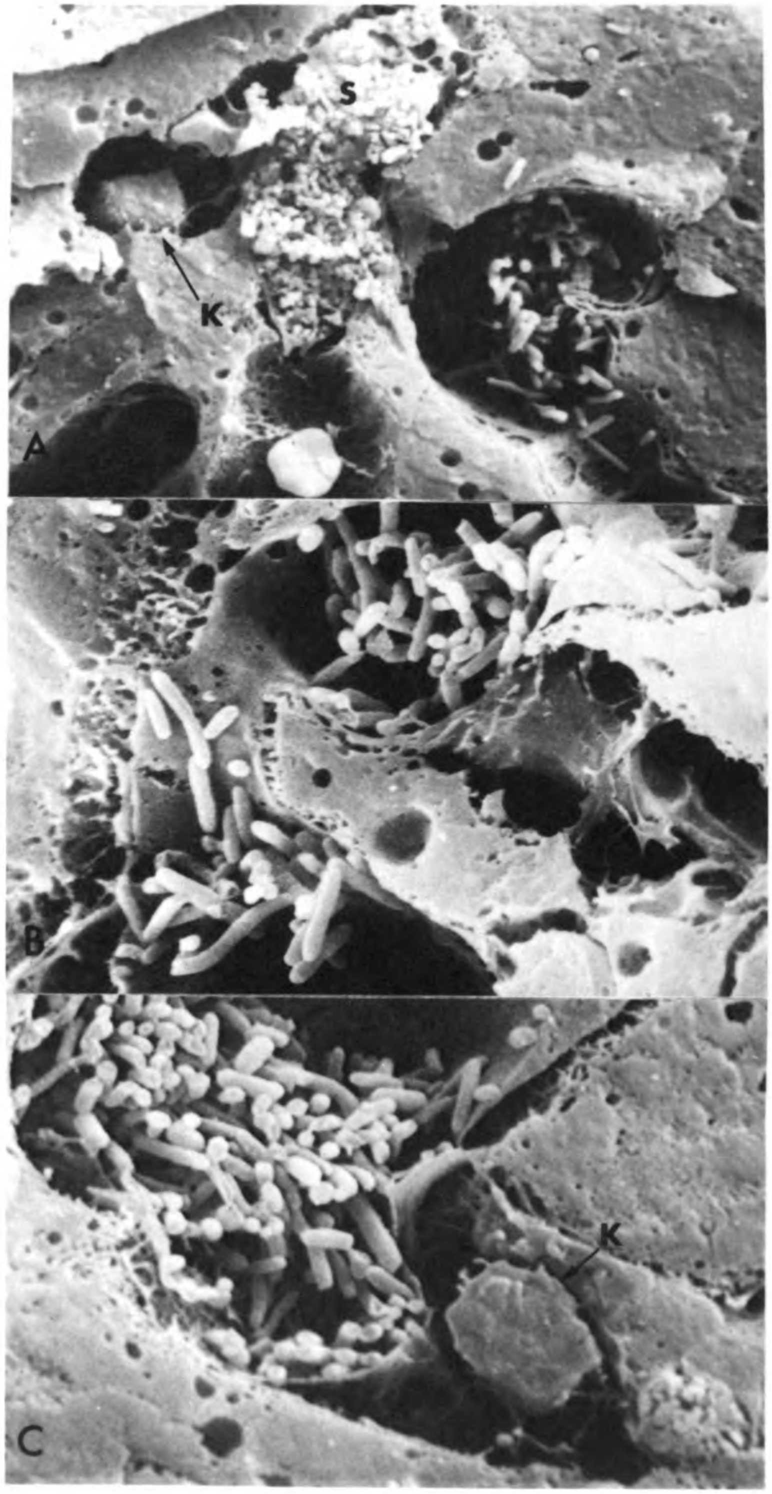

Figure 2

Figure 3. Rate of clearance of *S. typhimurium* from the blood of normal (●) and silica-treated (▲) mice. K = phagocytic index; $T\frac{1}{2}$ = biological half-life.

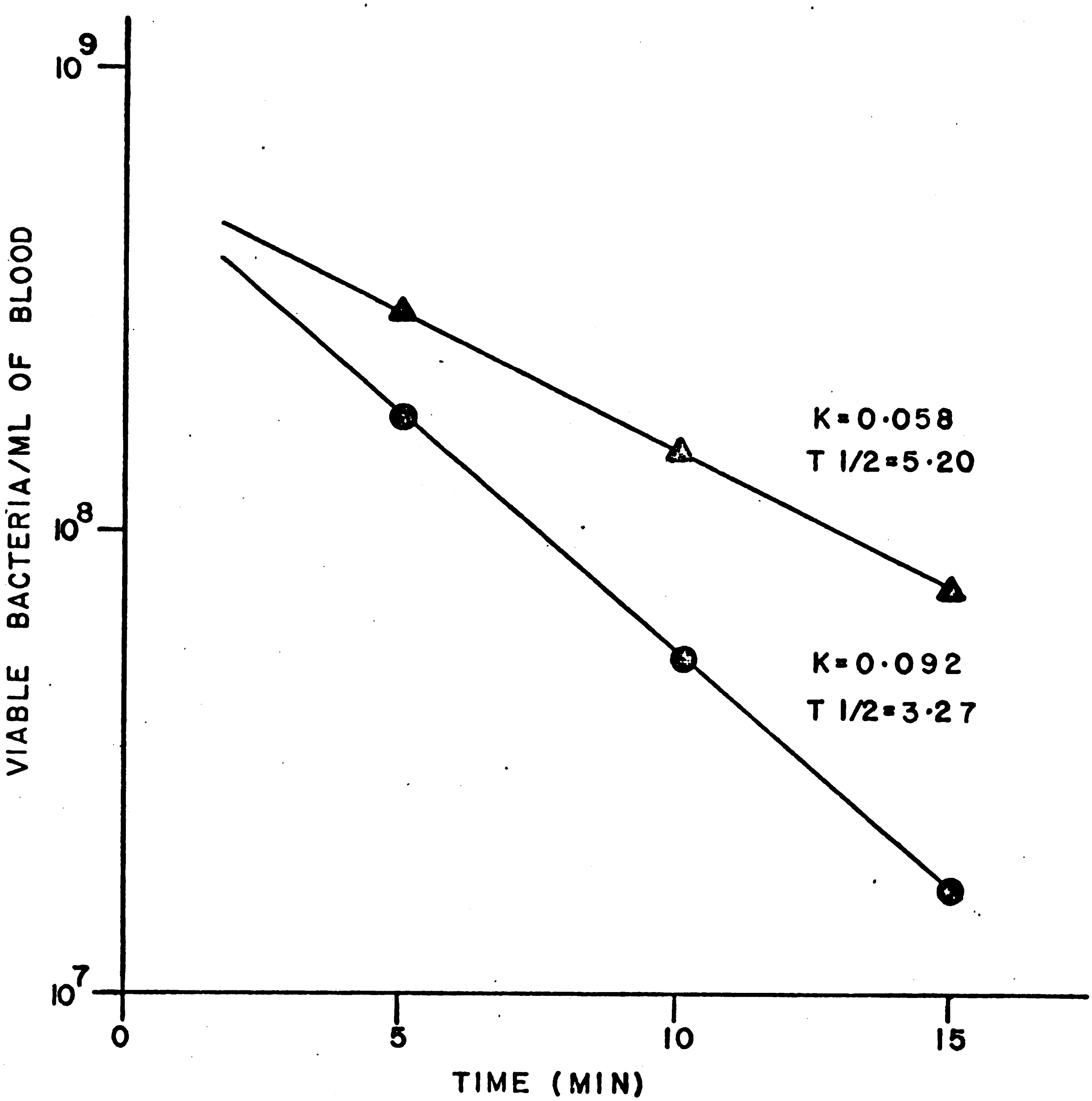
10^9
10^8
10^7
VIABLE BACTERIA/ML OF BLOOD
K=0·058
T 1/2=5·20
K=0·092
T 1/2=3·27
0
5
10
15
TIME (MIN)

Figure 3

points ($P<0.001$). The silica treatment suppressed the phagocytic index to 0.058 and increased T½ to 5.20 min.

The Effect of Crystalline Silica on Bacterial Killing and the Distribution of Viable *S. typhimurium* after I.V. Injection into Mice

Fifteen minutes after I.V. injection of 1-2 x 10^9 *S. typhimurium* into normal mice, 49.5% of the bacteria had been killed. Table 4a shows the distribution of the remaining viable organisms in the liver, spleen and carcass. In the liver, 40.2% were recovered while 7.5% were found in the carcass and 2.8% in the spleen. Silica-treated mice actually killed a higher percentage of bacteria (56.8% vs 49.5%) after 15 min, but the differences were not statistically significant. It should be noted that the distribution of remaining viable bacteria was very different. The majority of the viable bacteria (23.5%) were found in the carcass. The liver had only 12.7% and the spleen 6.9%.

After 30 min, in both normal and silica-treated mice, 81.5% of the injected bacteria were killed (Table 4b). Distribution of *S. typhimurium* in silica-treated animals was similar to the 15 min data.

Susceptibility to *S. typhimurium* Infection in DQ12 Silica-Treated Mice

Groups of normal mice and mice treated with either 3 or 10 mg of Dorënturp silica were challenged intravenously with either 2 x 10^5 or 2 x 10^3 *S. typhimurium*.

Table 4. Survival of *S. typhimurium* 15 min (4a) and 30 min (4b) after intravenous injection into normal and silica-treated mice[a]

Treatment	% Recovery in: Liver	Spleen	Carcass	Total Recovery (%)	Killing (%)
4a					
Control	40.2	2.8	7.5	50.5	49.5
Dorënturp silica	12.7[b]	6.9[c]	23.5[b]	43.2[c]	56.8[c]
4b					
Control	8.2	2.8	7.5	18.5	81.5
Dorënturp silica	3.7[b]	0.5[b]	14.3[b]	18.5[c]	81.5[c]

[a]Average of at least six separate experimental determinations.

[b]$P<0.001$.

[c]N.S.

One group of silica-treated animals at each test dose served as controls. No mice in the control groups died during the two weeks of the experiment. The mortality rate of *S. typhimurium* infected mice was followed for two weeks. Figure 4a shows that both doses of silica increased the rate of mortality slightly when mice were infected with 2×10^5 bacteria. A more dramatic difference was seen when mice were infected with 2×10^3 organisms (Figure 4b), where 83% of the normal mice

Figure 4. Susceptibility of normal and silica-treated mice to infection by 2×10^5 (4a) and 2×10^3 (4b) *S. typhimurium*. Normal control (■); 3 mg silica (▲); 10 mg silica (●).

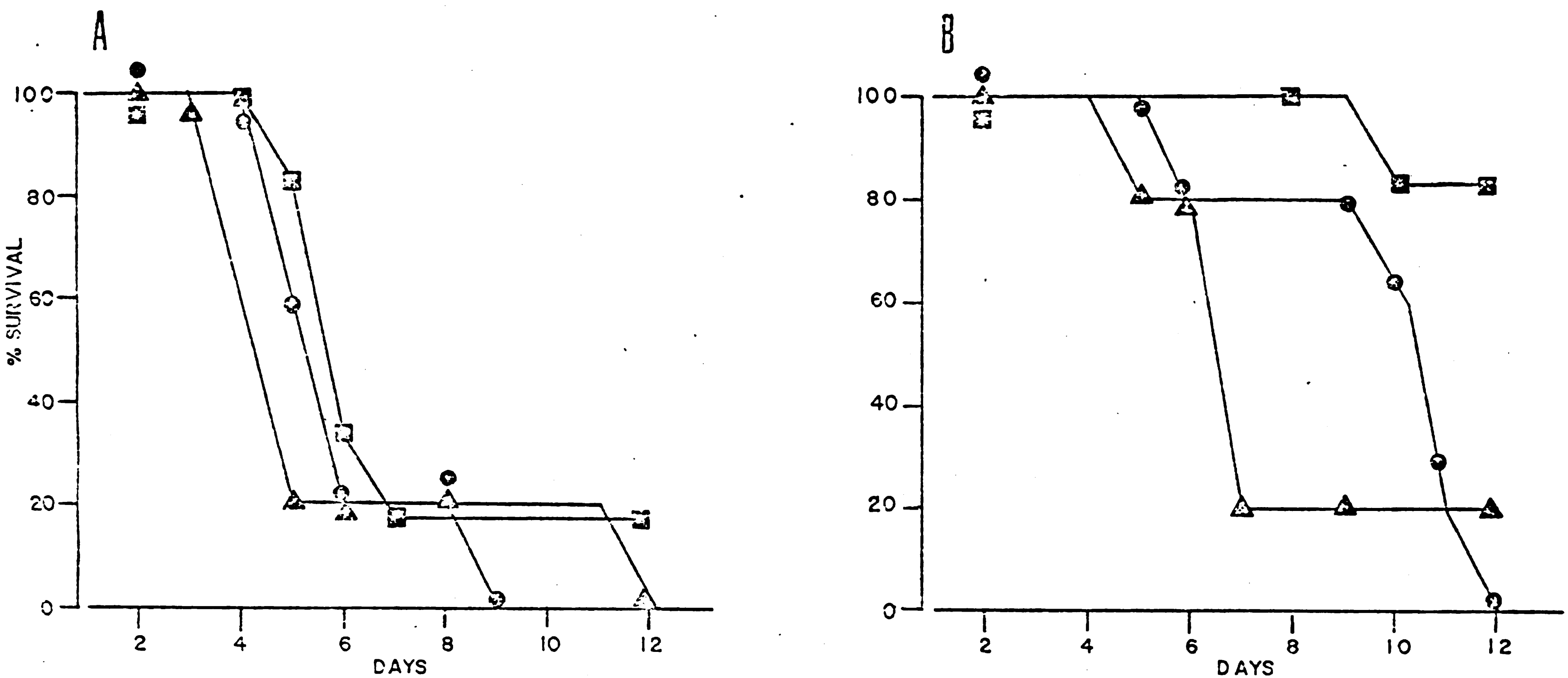

Figure 4

survived compared to only 20% in the 3 mg silica group and none survived in the 10 mg group. The LD_{50} for *S. typhimurium* in normal mice was calculated to be 8.0 x 10^4. The LD_{50} for silica-treated mice was less than 1 x 10^2 bacteria, indicating silica treatment enhanced susceptibility to *S. typhimurium* well over 100-fold.

Total White Blood Cell (WBC) and Differential Counts in Silica-Treated Mouse Blood

White blood cell counts and differential counts were made before and during the course of silica treatment. Table 5 shows that silica caused a leukocytosis and a lymphocytosis. Total PMN tripled from 2,000 before treatment to 6,000 after treatment. The total number of lymphocytes doubled from 9,487 to 20,371 after the injections of silica. Initially after the first injection of 3 mg of silica, there was a drop in numbers of PMN and lymphocytes. As the treatment proceeded, both cell types increased. While there were large increases in the numbers of PMN and lymphocytes, the proportion of each cell type in the blood stayed relatively constant except during the first day of silica treatment.

Table 5. White blood cell (WBC) and differential counts of silica-treated mice[a]

Mg Silica Injected	Cell Counts		Total WBC
	Lymphocytes	PMN	
0	9,487 (82.5%)	1,989 (17%)	11,476
3	4,094 (69%)	1,957 (31%)	6,051
6	14,292 (85%)	2,127 (15%)	16,419
10	20,371 (79%)	6,012 (21%)	26,383

[a]Average value of six separate experimental determinations.

DISCUSSION

In this study crystalline silica was used as an experimental tool to study the process of bacterial trapping in the isolated perfused liver. Moon et al. (57) recently demonstrated that bacterial clearance by the perfused liver is not synonymous with phagocytosis by Kupffer cells. *Salmonella typhimurium* was trapped in the liver sinusoids, giving a "log-jam" appearance. No bacterial killing occurred when whole blood was omitted from the perfusion media. In the presence of blood, 50% of the perfused *S. typhimurium* were killed. No killing occurred when bacteria were incubated with whole blood alone.

Bacterial trapping by the perfused liver may involve two distinct mechanisms, namely trapping by the Kupffer cells and by non-phagocytic endothelial cells. Scanning electron micrographs of silica-treated livers clearly demonstrated that DQ12 silica caused damage and destruction to Kupffer cells (cf. Figure 1) but had no other notable histotoxic effects on the liver. The destruction of liver Kupffer cells significantly decreased trapping of perfused *S. typhimurium* (cf. Table 3), but still, 31.3% of the bacteria were trapped. These data show that for maximal bacterial trapping by the perfused liver Kupffer cells must be viable. However, livers devoid of most functional Kupffer cells can still trap large numbers of bacteria in the sinusoids (cf. Figure 2), reinforcing the concept that bacterial trapping also involves non-Kupffer cell aspects of the liver.

No bacterial killing occurred in perfused livers from either normal or silica-treated mice. At all times 100% of the *S. typhimurium* were accounted for by total viable cells found in the effluent plus liver homogenates. This was expected since bacterial killing occurs only when humoral factors are present (14,36,40,41,57,75,76).

The liver is the major organ of the RES involved in clearing bacteria from the blood (75,76). The state of bacterial clearance *in vivo* could be evaluated by use of the perfused liver. Results of the *in vivo* experiments suggest that the isolated perfused liver was a true indicator of the state of bacterial clearance in the whole animal. Silica treatment significantly decreased carbon and bacterial clearance from the blood over a 15 min period. The phagocytic index (K) for bacteria dropped from 0.092 to 0.058 and the biological half-life increased from 3.27 min to 5.20 min. The distribution of the remaining viable bacteria varied greatly between silica-treated and normal mice, the majority of viable *S. typhimurium* recovered in silica-treated animals being in the carcass, while most bacteria in normal mice were found in the liver (cf. Table 4a,b). Decrease in bacterial clearance *in vivo* is consistent with results obtained in the perfused liver since, with depression of bacterial clearance by the liver *in vivo*, more organisms were found in other areas of the body.

Silica treatment was found to have a profound effect on mouse susceptibility to *S. typhimurium* infection (cf. Figure 4). Normal mice had an LD_{50} for *S. typhimurium* of 8.0×10^4. The LD_{50} for silica-treated mice was less than 1×10^2 organisms. This difference represents at least a 100-fold increase in susceptibility to *S. typhimurium* infection. These experiments are the first demonstration of enhancement of gram negative infection by silica. These data also emphasize the importance of clearing bacteria from the circulation by the RES, a function mainly done by the liver. The liver clears bacteria from the blood, localizing them to prevent spread of an infection to other areas of the body. As North has shown (66), blood monocytes accumulate in these infective foci. With impairment of blood clearance and possibly destruction of blood monocytes by silica, organisms remain in the blood longer and could form infective foci in other parts of the body. Bacteria may then multiply without antagonism by the RES.

The percent *S. typhimurium* killed *in vivo* after 15 and 30 min remained essentially the same in treated and normal mice. This similar bactericidal activity may be due to silica treatment causing an increase in numbers of blood PMN. It was found that total and differential cell counts from peripheral blood of silica-treated mice were elevated over the period of silica treatment, the total PMN tripling. Presumably, this leukocytosis explains why silica-treated animals were able to kill *S. typhimurium* as efficiently as normal nice.

While silica-treated mice still had normal initial bactericidal ability, they also had significantly increased susceptibility to *S. typhimurium* infection (cf. Figure 4a,b). These data strongly suggest that PMN are not the major defense mechanism against *S. typhimurium* infections, an interpretation consistent with Collins' work (18), suggesting that liver phagocytic cells may evolve cellular immunity which plays the ultimate role in host resistance to *Salmonella* infection.

LITERATURE CITED

LITERATURE CITED

1. Allison, A. C., J. S. Harington, and M. Birbeck. 1966. An Examination of the Cytotoxic Effects of Silica on Macrophage. J. Exp. Med. 124: 141-153.

2. Allison, A. C., and P. D. Hart. 1968. Potentiation by Silica of the Growth of *Mycobacterium tuberculosis* in Macrophage Cultures. Brit. J. Exp. Path. 49: 465-476.

3. Allison, A. C. 1971. Lysosomes and the Toxicity of Particulate Pollutants. Arch. Intern. Med. 128: 131-139.

4. Allison, A. C. 1970. On the Role of Macrophage in Some Pathological Processes, p. 422-444. In: *Mononuclear Phagocytes*. Van Furth Ed. Blackwell Sci. Publ., Oxford.

5. Baine, W. B., M. G. Koenig, and J. S. Goodman. 1974. Clearance of *Candida albicans* from the Bloodstream of Rabbits. Infect. Immun. 10: 1420-1425.

6. Benacerraf, B., E. Kivy-Rosenberg, M. M. Sebestyen, and B. W. Zweifach. 1959. The Effect of High Dose of X-Irradiation on the Phagocytic Proliferative and Metabolic Properties of the Reticulo-Endothelial System. J. Exp. Med. 110: 49-64.

7. Bennett, B., L. J. Old, and E. A. Boyse. 1964. The Phagocytosis of Tumor Cells *in vivo*. Transplantation 2: 183-202.

8. Berliner, D. L., and T. F. Dougherty. 1960. Influence of Reticuloendothelial and Other Cells on the Metabolic Fate of Steroids. Ann. N.Y. Acad. Sci. 88: 14-27.

9. Biozzi, G., B. Benacerraf, and B. N. Halpern. 1953. Quantitative Study of the Granulopectic Activity of the Reticuloendothelial System. II. A Study of the Kinetics of the Granulopectic Activity of the RES in Relation to the Dose of Carbon Injected: Relationships Between the Weight of the Organs and Their Activity. Brit. Exp. Path. 34: 441-457.

10. Biozzi, G., J. G. Howard, B. N. Halpern, C. Stiffel, and D. Mouton. 1960. The Kinetics of Blood Clearance of Isotopically Labelled *Salmonella enteritidis* by the Reticulo-Endothelial System in Mice. Immunology 3: 74-89.

11. Biozzi, G., C. Stiffel, B. N. Halpern, L. LeMinor, and D. Mouton. 1961. Measurement of the Opsonic Effect of Normal and Immune Sera on the Phagocytosis of *Salmonella typhi* by the Reticuloendothelial System. J. Immunol. 87: 296-300.

12. Biozzi, G., C. Stiffel, B. N. Halpern, and D. Mouton. 1963. Lack of Action of Serum Opsonins in Phagocytosis of Inert Particles by Cells of Reticuloendothelial System. Proc. Exp. Biol. Med. 112: 1017-1020.

13. Blickens, D. A., and N. R. DiLuizo. 1965. Metabolism of Methyl Plamitate, A Phagocytic and Immunologic Depressant and Its Influence on Tissue Lipids. J. Reticuloendothel. Soc. 2: 60-74.

14. Bonventre, P. F., and E. Oxman. 1965. Phagocytosis and Intracellular Disposition of Viable Bacteria by the Isolated Perfused Rat Liver. J. Reticuloendothel. Soc. 2: 313-325.

15. Bryson, G., and F. Bischoff. 1967. Silicate-Induced Neoplasms. Progr. Exp. Tumor Res. 9: 77-164.

16. Bull, C. G. 1915. The Fate of Typhoid Bacilli when Injected Intravenously into Rabbits. J. Exp. Med. 22: 475-483.

17. Carr, I. 1973. The Macrophage in Iron and Lipid Metabolism, p. 99-105. In: *The Macrophage A Review of Ultrastructure and Function.* Academic Press, New York.

18. Collins, F. M. 1974. Vaccines and Cell-Mediated Immunity. Bact. Rev. 38: 371-402.

19. Comolli, R., and A. Perin. 1963. *In vitro* Action of Silicogenic and Non-Silicogenic Dusts on Macrophage Metabolism. Proc. Soc. Exp. Biol. Med. 113: 289-293.

20. Cummings, D. E. 1929. Studies on Experimental Pneumonokoniosis. The Separation of Particulate Matter Smaller than Screen Size into Graded Fractions. J. Indust. Hyg. 11: 245-256.

21. Curran, R. C., and E. V. Rowsell. 1958. The Application of the Diffusion-Chamber Technique to the Study of Silicosis. J. Path. Bact. 76: 561-568.

22. DiLuzio, N. R. 1960. Reticuloendothelial Involvement in Lipid Metabolism. Ann. N.Y. Acad. Sci. 88: 244-251.

23. DiLuzio, N. R., and W. R. Wooles. 1964. Depression of Phagocytic Activity and Immune Response by Methyl Palmitate. Amer. J. Physiol. 206: 939-943.

24. Evans, R., and P. Alexander. 1972. Role of Macrophages in Tumor Immunity. I. Co-Operation Between Macrophages and Lymphoid Cells in Syngeneic Tumor Immunity. Immunology 23: 615-626.

25. Filkins, J. P., R. E. Chase, and J. J. Smith. 1965. Characteristics of a Plasma Factor Governing Carbon Phagocytosis in the Isolated Perfused Rat Liver. J. Reticuloendothel. Soc. 2: 287-295.

26. Filkins, J. P., and J. J. Smith. 1965. Plasma Factor Influencing Carbon Phagocytosis in the Isolated Perfused Rat Liver. Proc. Soc. Exp. Biol. Med. 119: 1181-1184.

27. Filkins, J. P. 1971. Hepatic Lysosomes and the Inactivation of Endotoxin. J. Reticuloendothel. Soc. 9: 480-490.

28. Friedman, H. 1972. The Role of the Reticuloendothelial System in Infection and Immunity - General Aspects. J. Reticuloendothel. Soc. 11: 445-449.

29. Gardner, L. U. 1923. Studies on the Relation of Mineral Dusts to Tuberculosis. Am. Rev. Tuberc. 7: 344-357.

30. Goldfischer, S., A. B. Novikoff, A. Albala, and L. Biempica. 1970. Hemoglobin Uptake by Rat Hepatocytes and Its Breakdown Within Lysosomes. J. Cell Biol. 44: 513-529.

31. Gordon, L. E., D. B. Cooper, and C. P. Miller. 1955. Clearance of Bacteria from the Blood of Irradiated Rabbits. Proc. Soc. Exp. Biol. Med. 89: 577-579.

32. Grogan, J. B. 1969. Alterations in Phagocytic Function of Rats after Treatment with Antilymphocyte Serum. J. Reticuloendothel. Soc. 6: 411-418.

33. Gye, W. E., and W. J. Purdy. 1922. Poisonous Properties of Colloidal Silica. I. The Effects of the Parenteral Administration of Large Doses. Brit. J. Exp. Path. 3: 75-85.

34. Hope, J., and J. V. Friend. 1969. Preliminary Scanning Electron Microscopic Observation on the Effect of Silica Dusts on Cultured Macrophages. Micron. 1: 310-324.

35. Howard, J. G., and A. C. Wardlaw. 1958. The Opsonic Effect of Normal Serum on the Uptake of Bacteria by the Reticulo-Endothelial System. Perfusion Studies with Isolated Rat Liver. Immunology 1: 338-352.

36. Howard, J. G. 1961. The Reticuloendothelial System and Resistance to Bacterial Infection. Scot. Med. J. 6: 60-82.

37. Hyman, C., and R. L. Paldino. 1960. Possible Role of the Reticuloendothelial System in Protein Transport. Ann. N.Y. Acad. Sci. 88: 232-239.

38. Jenkin, C. R., and D. Rowley. 1961. The Role of Opsonins in the Clearance of Living and Inert Particles by Cells of the Reticuloendothelial System. J. Exp. Med. 114: 363-374.

39. Jeunet, F. S., and R. A. Good. 1967. Reticuloendothelial Function in the Isolated Perfused Liver. I. Study of Rates of Clearance, Role of a Plasma Factor, and the Nature of RE Blockade. J. Reticuloendothel. Soc. 4: 351-369.

40. Jeunet, F. S., W. Cain, and R. A. Good. 1968. Differential Recognition of *Brucella* Organisms by Kupffer Cells: Studies with Isolated Perfused Liver. Proc. Exp. Biol. Med. 129: 187-190.

41. Jeunet, F. S., W. A. Cain, and R. A. Good. 1969. Reticuloendothelial Function in the Isolated Perfused Liver. III. Phagocytosis of *Salmonella typhosa* and *Brucella melitensis* and the Blockade of the Reticuloendothelial System. J. Reticuloendothel. Soc. 6: 391-410.

42. Keller, R. 1973. Cytostatic Elimination of Syngeneic Rat Tumor Cells *in vitro* by Nonspecifically Activated Macrophages. J. Exp. Med. 138: 625-644.

43. Keller, R. 1974. Mechanisms by which Activated Normal Macrophages Destroy Syngeneic Rat Tumor Cells *in vitro*. Immunology 27: 285-298.

44. Kessel, R. W. I., L. Monaco, and M. A. Marchisio. 1963. The Specificity of the Cytotoxic Action of Silica-A Study *in vitro*. Brit. J. Exp. Path. 44: 351-364.

45. Kettle, E. H. 1926. Experimental Silicosis. J. Indust. Hyg. 8: 491-495.

46. Kierszenbaum, F., E. Knecht, D. B. Budzko, and M. C. Pizzimenti. 1974. Phagocytosis: A Defense Mechanism Against Infection with *Trypanosoma cruzi*. J. Immunol. 112: 1839-1844.

47. King, E. J. 1947. Solubility Theory of Silicosis - A Critical Study. Occup. Med. 4: 26-49.

48. Larson, C. L., R. N. Ushijima, R. E. Barker, M. B. Barker, and C. A. Gillespie. 1972. Effect of Normal Serum and Anti-Lymphocyte Serum on Friend Disease in Mice. J. Nat. Cancer Inst. 48: 1403-1407.

49. Levy, M. H., and E. F. Wheelock. 1975. Effects of Intravenous Silica on Immune and Non-Immune Functions of the Murine Host. J. Immunol. 115: 41-48.

50. Lirenman, D. S., A. J. Fish, and R. A. Good. 1967. Lack of a Serum Factor in Preventing Blockade of the Reticuloendothelial System in the Rabbit. J. Reticuloendothel. Soc. 4: 34-42.

51. Lotzova, E., and G. Cudkowicz. 1974. Abrogation of Resistance to Bone Marrow Grafts by Silica Particles. Prevention of the Silica Effect by the Macrophage Stabilizer Poly-2-Vinylpyridine N-Oxide. J. Immunol. 113: 798-803.

52. Manwaring, W. H., and H. C. Coe. 1916. Endothelial Opsonins. J. Immunol. 1: 401-408.

53. Manwaring, W. H., and W. Fritschen. 1923. Study of Microbic-Tissue Affinity by Perfusion Methods. J. Immunol. 8: 83-89.

54. Marks, J., and G. Nagelschmidt. 1959. Study of the Toxicity of Dust with Use of the *in vitro* Dehydrogenase Technique. Arch. Industr. Hyg. and Occ. Med. 20: 383-389.

55. Miller, S. D., and A. Zarkower. 1974. Alterations of Murine Immunologic Responses After Silica Dust Inhalation. J. Immunol. 113: 1533-1543.

56. Miller, S. D., and A. Zarkower. 1976. Silica-Induced Alterations of B Lymphocyte Immunocompetence: A Possible Mechanism. J. Reticuloendothel. Soc. 19: 47-61.

57. Moon, R. J., R. A. Vrable, and J. A. Broka. 1975. In situ Separation of Bacterial Trapping and Killing Functions of the Perfused Liver. Infect. Immun. 12: 411-418.

58. Mouton, D., G. Biozzi, Y. Bouthillier, and C. Stiffel. 1963. Phagocytosis of *Salmonella* by Reticuloendothelial Cells of New-Born Piglets Lacking Natural Antibody. Nature 197: 706.

59. Murray, I. M. 1963. The Mechanism of Blockade of the Reticuloendothelial System. J. Exp. Med. 117: 139-147.

60. Murray, I. M. 1963. Clearance Rate in Relation to Agglutinins for Gelatin-Stabilized Colloid in the Rat. Amer. J. Physiol. 204: 655-659.

61. Nabors, C. J., Jr., D. L. Berlinger, and T. F. Dougherty. 1967. The Function of Hepatic Reticuloendothelial Cells in Steroid Hormone Biotransformation. J. Reticuloendothel. Soc. 4: 237-253.

61. Nadler, S., and S. Goldfischer. 1970. The Intracellular Release of Lysosomal Content in Macrophages that have Ingested Silica. J. Histochem. Cytochem. 18: 368-371.

63. Nash, T., A. C. Allison, and J. S. Harington. 1966. Physicochemical Properties of Silica in Relation to its Toxicity. Nature 210: 259-261.

64. Nicol, T., R. S. Snell, and D. L. J. Bilbey. 1956. Effect of Cortisone on the Defense Mechanism of the Body. Brit. Med. J. 2: 800-801.

65. Normann, S. J., and E. P. Benditt. 1965. Function of the Reticuloendothelial System. I. Study on the Phenomenon of Carbon Clearance Inhibition. J. Exp. Med. 122: 693-707.

66. North, R. J. 1970. The Relative Importance of Blood Monocytes and Fixed Macrophage to the Expression of Cell-Mediated Immunity to Infection. J. Exp. Med. 132: 521-534.

67. Page, R. C., P. Davies, and A. C. Allison. 1974. Participation of Mononuclear Phagocytes in Chronic Inflammatory Diseases. J. Reticuloendothel. Soc. 15: 413-438.

68. Pearsall, N. N., and R. S. Weiser. 1968. The Macrophage in Allograft Immunity. I. Effects of Silica as a Specific Macrophage Toxin. J. Reticuloendothel. Soc. 5: 107-120.

69. Pernis, B., and F. Paronetto. 1962. Adjuvant Effect of Silica (Tridymite) on Antibody Production. Proc. Soc. Exp. Biol. Med. 110: 390-393.

70. Pisano, J. C., and N. R. DiLuzio. 1970. Effect of Anti-Kupffer Cell Serum on Phagocytosis and Humoral Antibody Formation. Infect. Immun. 2: 448-452.

71. Pisano, J. C., J. T. Patterson, and N. R. DiLuzio. 1969. Depression of Phagocytic Activity of the Reticuloendothelial System by Antilymphocytic Serum (ALS). Proc. Soc. Exp. Biol. Med. 132: 517-523.

72. Razumov, M. I., B. K. Skirko, and A. YU. Grubina. 1963. Comparison of the Silicogenic Effects of Various Quartz Preparations. Fed. Proc. Transl. Suppl. 22: T502-T506.

73. Rios, A., and R. L. Simmons. 1972. Poly-2-Vinyl-Pyridine N-Oxide Reverses the Immunosuppressive Effects of Silica and Carrageenan. Transplantation 13: 343-345.

74. Robock, K. 1973. Standard Quartz DQ12 <5 μM for Experimental Pneumoconiosis Research Projects in the Federal Republic of Germany. Ann. Occ. Hyg. 16: 63-66.

75. Rogers, D. E. 1960. Host Mechanisms Which Act to Remove Bacteria from the Blood Stream. Bact. Rev. 24: 50-66.

76. Saba, T. M. 1970. Physiology and Physiopathology of the Reticuloendothelial System. Arch. Intern. Med. 126: 1031-1052.

77. Sawyer, R. 1976. Personal communication.

78. Scheel, L. D., E. Fleisher, and F. W. Klemperer. 1953. Toxicity of Silica. I. Silica Solutions. Arch. Indust. Hyg. and Occup. Med. 8: 564-573.

79. Scheel, L. D., B. Smith, J. VanRiper, and E. Fleisher. 1954. Toxicity of Silica. II. Characteristics of Protein Films Adsorbed by Quartz. Arch. Indust. Hyg. and Occup. Med. 9: 29-36.

80. Sharabaugh, R. J., and J. B. Grogan. 1969. Suppression of the Reticuloendothelial Function in the Rat with Cyclophosphamide. J. Bact. 100: 117-122.

81. Stiffel, C., D. Mouton, and G. Biozzi. 1970. Kinetics of Phagocytic Function of Reticuloendothelial Macrophage *in vivo*, p. 335-381. In: *Mononuclear Phagocytes*. Van Furth Ed. Blackwell Publ., Oxford.

82. Stuart, A. E., G. Biozzi, C. Stiffel, B. N. Halpern, and D. Mouton. 1960. The Stimulation and Depression of Reticuloendothelial System Phagocytic Function by Simple Lipids. Brit. J. Exp. Path. 41: 599-604.

83. Thomas, L. 1953. Cortisone and Infection. Ann. N.Y. Acad. Sci. 56: 799-814.

84. Turner, K. J., J. Papadimitriou, R. Hackshaw, and J. D. Wetherall. 1975. Experimental Aspergillosis in Normal Rats Infected Intravenously. J. Reticuloendothel. Soc. 17: 300-312.

85. Vigliani, E. C., and B. Pernis. 1963. Immunological Aspects of Silicosis. Adv. Tuberc. Res. 12: 230-279.

86. Vorwald, A. J., and A. B. Delahant. 1938. The Influence of Silica on the Natural and Acquired Resistance of the Tubercle Bacillus. Amer. Rev. Tuberc. 38: 347-362.

87. Vorwald, A. J., M. Dworski, P. C. Pratt, and A. B. Delahant. 1954. BCG Vaccination in Silicosis. An Experimental Study of the Influence of Inhaled Quartz Dust Upon Infection by BCG (Aronson), H37Ra and *M. marinum* Strains of Tubercle Bacilli. Amer. Rev. Tuber. 69: 776-789.

88. Wagner, H. N., Jr., M. Ilo, and R. B. Hornick. 1963. Studies on the RES. II. Changes in the Phagocytic Capacity of the RES in Patients with Certain Infections. J. Clin. Invest. 42: 427-434.

89. Wardlaw, A. C., and J. G.Howard. 1959. A Comparative Survey of the Phagocytosis of Different Species of Bacteria by Kupffer Cells. Brit. J. Exp. Path. 40: 113-117.

90. Wiener, J., T. S. Cotterell, W. Margaretten, and D. Spiro. 1967. An Electron Microscopic Study of Steroid Induced Reticuloendothelial Blockade. Amer. J. Path. 50: 187-201.

91. Wiener, J., D. Spiro, and W. Margaretten. 1964. An Electron Microscopic Study of Reticuloendothelial System Blockade. Amer. J. Path. 45: 783-803.

92. Wiener, J. 1967. Fine Structural Aspects of Reticuloendotehlial Blockade, p. 85-95. In: *Reticuloendothelial System and Ahterosclerosis*. N. R. DiLuzio and R. Paoletti Ed. Plenum Press, New York.

93. Wilcoxon, F., and R. A. Wilcox. 1949. Some Rapid Approximate Statistical Procedures. American Cyanamid Co., New York.

94. Zarling, J. M., and S. S. Tevethia. 1973. Transplantation Immunity to SV40 Transformed Cells in Tumor Bearing Mice. II. Evidence for Macrophage Participation at the Effector Level of Tumor Cell Rejection. J. Nat. Cancer Inst. 50: 149-157.

95. Zisman, B., M. S. Hirch, and A. C. Allison. 1970. Selective Effects of Anti-Macrophage Serum, Silica and Anti-Lymphocyte Serum on Pathogenesis of Herpes Virus Infection of Young Adult Mice. J. Immunol. 104: 1155-1159.

96. Zisman, B., E. F. Wheelock, and A. C. Allison. 1971. Role of Macrophages and Antibody in Resistance of Mice Against Yellow Fever Virus. J. Immunol. 107: 236-243.

97. Rager-Zisman, B., and A. C. Allison. 1973. The Role of Antibody and Host Cells in the Resistance of Mice Against Infection by Coxsackie B-3 Virus. J. Gen. Virol. 19: 329-338.