LOCAL IMMUNOLOGY OF THE COLLAPSED LUNG

A Thesis

by

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ABSTRACT

The clinical importance of pulmonary infection in the post-operative or traumatized surgical patient is well known. Likewise, its close association with multiple organ failure is becoming increasingly known. In order to examine some of the etiologies, we designed a model which would reflect a common clinical problem thought to predispose the lung to infections, that is, atelectasis. This necessitated a thorough review of the lung defense systems and particularly, the local lung immunology. In our experiments, we found that bacterial clearance in the atelectatic lung was depressed versus the control. By cannulation of the right lymphatic duct, we demonstrated that infection and atelectasis created a high permeability edema. Our morphologic and function studies of the alveolar macrophage revealed it is uniquely adaptable to the relative hypoxia of collapsed airways. We found that alveolar macrophages from the atelectatic lung had depressed chemotaxis, but increased phagocytosis and bactericidal capacity. The results implicate a mechanical change rather than a cellular, immunologic one as primary reason for decreased bacterial clearance.

We examine and discuss the bacterial aspects of setting up a laboratory for these functional studies.

ABSTRACT

L'importance clinique des infections pulmonaires chez les patients en état post-opératoire est bien connue. De même, son association avec insuffisance de multiples organes est reconnue de plus en plus. Afin d'étudier ces phenomènes, nous avons devéloppé un modèle que représénterait un problème clinique commun reconnu pour prédisposer les poumons aux infections, c'est-à-dire, atelectosie. Ceci a nécessité une revue complète des systèmes de défense des poumons et particulièrement l'immunologie pulmonaire locale. Lors de nos expériences, nous avons constaté que l'élimination bactérienne dans les poumons atélectotiques était diminuée, lorsque comparée a un contrôle normal. En cannulant, le canal lymphatique droit, nous avons démontré que l'infection et l'atelectosie provoquaient un oedème pulmonaire non-cardiogène. Nos études morphologique et physiologiques des macrophages alvéolaires ont révélé que ceux-ci sont uniquement adaptables à la carence relative d'oxygène dans les voies respiratoires effondrées. Nous avons démontré que la motilité des macrophages alvéolaires retrouvés dans les poumons atélectotiques était diminuée mais leur activité phagocytique et bactéricide était augmentée. Les résultats obtenus tendent à montrer que la dimunution de l'élimination bactérienne provient plus d'une modification méoanique que cellulaire ou immunologique. Nous examinons et discutons les aspects techniques de la mise sur pied d'un laboratoire pour ces études functionelles.

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PERSPECTIVE

> They (bacteria) will invade and replicate if given the chance, and some of them will get into our deepest tissues and set forth in the blood, but it is our response to their presence that makes the disease. Our arsenals for fighting off bacteria are so powerful, and involve so many different defense mechanisms, that we are in more danger from them than from the invaders. We live in the midst of explosive devices; we are mined (172).

These words apply to normal individuals in normal situations; but surgical patients are not normal nor is the situation. Indeed, as we learn more about the effects of any of man's interventions on man, "normal" seems rarer and more distant. And nowhere is this more true than in the lung.

The lung is a unique "internal" organ, with the greatest exposure to the external environment. We discover with increasing regularity that the environment contains large numbers of microbes, large numbers of particles of inorganic and organic plant material; and industrial wastes either in gas or solid states.

It is surprising there is not more morbidity and mortality in the normal individual who is constantly bombarded by these particles, breathing an average of 29,000 times a day for a total exchange of roughly 20,160,000 mls of air. An estimated 10-20,000 microorganisms are inhaled per day in an average man of which the majority are airborne bacteria and fungi (88).

Unfortunately, we are now discovering that even normal respiratory systems are greatly affected in numerous situations. The debilitation and lethality of some of these substances, particularly microbes, is even greater in the immunocompromised person, or in persons whose defenses are altered by some intervention.

The congenitally afflicted, the malnourished, the traumatized all are at higher risk for respiratory disease that could be fatal. For surgeons, the latter two are of particular interest as the two are often combined in a patient who is unable to take adequate nourishment, and who must undergo surgical intervention for a life-threatening disease process. Even the patient with less urgent diseases that require elective surgical intervention have insults and alterations on their respiratory system that can have led to serious complications. In these groups, our interest is often focused on the antimicrobial capability of the upper and lower respiratory tract for good reason.

A recent study from Louisville (50) places the magnitude of the problem in perspective, as they examined the causes of post-traumatic pulmonary insufficiency in man. Their study involved 399 patients admitted to their surgical service with an illness or injury involving abdominal disease, flail chest, neurologic injury, or long bone fracture with severity ranging from appendicitis to multiple gunshot wounds

and ruptured aortic aneurysms. Patient charts were reviewed and a statistical analysis was made of the association of post-traumatic pulmonary insufficiency with the illnesses. They found that 44 or 11% developed respiratory failure, as defined by severely impaired pa02 and the need for prolonged ventilatory assistance. As well, 90 or 25% required mechanical ventilation assistance with endotracheal intubation; and virtually all 399 patients received some form of respiratory therapy in the form of oxygen therapy. The mortality rate for patients with post-traumatic pulmonary insufficiency was 50%. There was a clear association of sepsis, hypovolemic shock and massive fluid therapy with insufficiency, but sepsis was the most common single factor associated with pulmonary insufficiency. Indeed, over 90% of these patients were septic.

In this study, although the primary septic focus was extra-pulmonary in 55%, essentially all the patients with pulmonary insufficiency eventually developed pneumonic infiltrates with pathogenic organisms recoverable from their sputa. In fact, 26 eventually succumbed as a result of the respiratory infections. The authors tender two mechanisms for the localization of infection in the lungs. First, there was a group of patients (45%) with a primary pneumonic septic focus, exacerbated by lung injury as well as intubation which removed an effective cough reflex; and second, a remote septic focus seeded the lung during filtration of the systemic circulation forming septic emboli in the other group (50%).

This study epitomizes the consequences of a relatively new concept, and that is "multiple organ failure", in which it appears that pulmonary insufficiency (or adult respiratory distress syndrome, or shock lung) may represent the final common pathway. A mortality rate of 59% is adequate proof of its clinical importance. The study also epitomizes the current concept and understanding of the disease process whose pathogenesis is still not well understood, and contains many important gaps, particularly at the cellular level.

The clinical importance as well as the gaps as incentives, we focused our attention on a common clinical observation that collapsed or atelectatic lungs, for whatever reasons, is at greatly increased risk for infections, excluding ventilation complications. As well, there appears to be a strong relationship between pulmonary edema, from either heart failure, or the so-called "shock lung", and depressed antimicrobial capacity. This depressed state goes far beyond the general association with a chronic and debilitating state such as congestive heart failure. Of course, there is, in all of this, the "chicken-or-the-egg phenomenon"; that may in reality be a moot point. The consequences of infection and overhydration motivates our aggressive use of blow bottles, chest physio, and early ambulation in the surgical patient; and also the judicious titration of intravenous fluids for adequate, not over, hydration. Other tools we have at our disposal are ventilatory assistance, caloric and protein

replacement using parenteral hyperalimentation, and of course, an assortment of pharmacologic agents to kill microbes and remove fluid from the intravascular space.

Inherent in these clinical observations and beliefs is the question whether atelectasis and pulmonary edema do, in fact, predispose the lung to microbial infection; and even larger questions as to possible pathophysiology. This includes some firm knowledge of anatomical defenses of the upper respiratory tract, and some not-so-firm knowledge of the fluid dynamics in the lung, and some infant knowledge of the local immunologic system in the lung.

We, therefore, set out to develop both a model and a laboratory capable of investigating some of these physiologic and immunologic questions. We will discuss the developmental aspect of this work, literature review of these subjects, and pitfalls that we encountered in tests and interpretation - the latter again reflecting the early state of the art. Likewise, we will stress the ongoing nature of this work and focus on several areas that we think are key to a greater understanding of local lung immunology.

We elected to simplify the functional measurement of antimicrobial activity to be the same as bacterial clearance, a reproducible testing method whose assumption is valid for most of the common pathogens. The rate of bacterial

clearance from the lung is thought to depend on four factors: the rate of phagocytosis, the pulmonary alveolar macrophage intracellular killing, the physical removal of bacteria by mucociliary function, and the rate of bacterial multiplication (80). Our study, therefore, began looking at these and other variables, after first establishing whether our original question was valid, does atelectasis predispose to bacterial infections? To fully examine this problem, a model had to be developed that satisfied some important criteria. It must utilize an animal as close to the human as is feasible, have variables imposed that are extreme enough to provide us with clear-cut reproducible data, and have a control built in at all stages. Finally, the model must be flexible enough to allow input variation to obtain data of any changes at the structural, cellular and metabolic levels.

FILTRATION AND DETOXIFICATION

> Disease usually results from inconclusive negotiations for symbiosis, an overstepping of the line by one side or the other, a biologic misinterpretation of borders (172).

The body has developed a formidable arsenal for defending itself from pathogens, bacteria, fungi, viruses, and protozoans for a very good reason- they outnumber us. In fact, it is estimated that approximately 10^{14} bacteria inhibit our gastro-intestinal tract, predominantly our large bowel although there are estimated to be only 10^{13} cells in our entire body. As a reflection of the bacterial influence on our development, our mitochondria are thought now to have evolved from bacteria rather than from our own cells.

In general, we live in a symbiotic relationship with infection being the exception rather than the rule. Our body over the years developed an array of defenses which have been arbitrarily divided into local, or those that prevent invasion by the microbes; and systemic, those that are activated after the infection has taken place. In the examination of the immunology of the lung, we will focus on the local defense capabilities, that is, the preventive defense actions prior to actual tissue invasion of the pathogens.

AIRWAY FILTRATION:

Air filtration by the respiratory system is an important defense mechanism of the lung gainst inhaled toxins and pathogens. The contact area at risk is 80 to 90 meters

squared, the alveoli surface area, and represents an area of contact between the body and external environment that is 40 times greater than the surface area of skin. A very fine review by Newhouse, as well as several other studies provides the basis for this section review (30,42,46,128).

The removal of particulate and gaseous materials occurs in the naso-pharynx in the normal individual. Nasal hairs and mucus trap many of the inhaled particulate matter. Gases that are primarily absorbed are the highly soluble, such as ozone, ammonia, and sulphur dioxide. Impaction of any "escaped" particle may then occur in the lower naso-pharynx where an acute right angle is made, allowing inertial impaction against strategically placed lymphoid tissue, like the tonsils or adenoids.

In the mouth, the tissues of the naso-pharynx and oro-pharynx are protected by the physical barrier of the mucous epithelium and by approximately a liter production of saliva per day, which in itself has a mechanical cleansing action. Likewise, saliva contains lysozyme, that is capable of splitting the linkage in bacterial cell walls; and as well saliva contains a secretory immunoglobulin IGA which in itself is capable of neutralizing viruses and preventing adhesion of bacteria.

The rare larger particles that reach the coryna or the first two bronchial divisions are removed after impaction at this level. Particles .2 to 5 micron size are cleared by sedimentation. This results from gravitational force in

the slow laminar flow area of the lung, that is, the 15 to the 23rd bronchial generation, where the sudden expansion in relative airway cross-sectional area accounts for reduction in flow.

For even smaller particles, .1 micron size, Brownian Movement facilitates extraction by impaction on mucous surfaces which may take place at any level in the lung. This particle size is very important epidemiologically as these tend not to settle on the ground or external surface areas, and therefore, are the most commonly inhaled.

Size and shape of particles help dictate the level or extent of invasion. Asbestos fibers, for example, although up to 300 microns in length are very narrow, 1-2 microns in width, and are therefore found in the alveoli far distal, by aligning themselves parallel to the direction of the airway (128). Bronchoconstriction in the airways, either physiologic or pathologic, may prevent distal alveoli exposure. One reflex is vagally mediated for smooth muscle contraction, which may be stimulated by both mechanical and immunologic factors. The constriction causes increased air resistance, but little change in pulmonary compliance so that the number of ventilated units remains stable, in contrast to airways pathologically involved in say COPD, which does cause V/Q changes. Studies of coal miners who are cigarette smokers, has demonstrated that alveoli distal to segments of bronchitis are "protected" from pneumoconiosis (128,42).

AIRWAY CLEARANCE:

Once material has been inspired or aspirated, the mucociliary system is vital to adequate particulate clearance from lung segments, but often adversely affected for iatrogenic reasons. The system begins with the production of the mucopolysaccharide by the goblet or mucous secreting glands in the large airways. When infected, the mucus tends to lose elasticity, to become tenacious, causing inspissated plugs thought to be one of the mechanisms in the formation of atelectasis post-operatively. The mucus itself contains immunoglobulins, particularly secretory IgA as well as lysosomal enzymes (12). Mechanically, the mucus provides a 7 micron layer throughout the upper airways, thereby humidifying inspired air, and at the same time preventing water loss. Areas such as where intubation is performed, have interruption of this membrane with inflammation and activation of the complement cascade in the particular denuded area.

The cilia are an integral portion of the system providing transportation for the layer of mucus which acts as a rug or escalator for particle removal. The cilia are made up of microtubules that beat at between 1,000 and 1,500 cycles a minute, utilizing the oxidative-phosphorylation pathways for energy. Their activity is felt similar to cardiac contractility; in fact, it has been shown, that digitalis improves the cilia function in hypoxic cats (128). Velocity in the small airways is 1 to 2 mm per minute, and in large airways 5 to 20 mm per minute - virtually all material deposited on normal ciliated epithelium is removed in less

than 24 hours. Substances known to be toxic for cilia are: oxygen in high concentrations, cigarette smoke, sulphur dioxide, pentobarbitol (in sheep), alcohol, and various viral infections. Also, a recently described syndrome called Cartagener's Syndrome has absent cilia function, as well as hypomotility of spermatozoa with infertility (42,105,128).

The mucociliary system may have congenital, metabolic, and toxic malfunctioning effects; and two recent studies now implicate age and iatrogenic care. Puchelle examined the effect of age and bronchial mucociliary clearance using radiolabled resin washout curves in 19 healthy, non-smoking male subjects, ages 21 to 69 (140). She found the clearance rate significantly lower in older subjects, particularly greater than 53 years. However, the large inter and intra-individual coefficients of variation led the author to conclude that factors other than age may also have an effect on clearance. Another recent study found that tracheo-bronchial suctioning in sheep depressed mucous transport (105). The histologic effects were varying degrees of mucosal denudation and edema of the lamina propria with extravasation of red blood cells and dense infiltration of polys and lymphocytes. Importantly, three types of catheters were examined in this process, Bard side-hole, the Aero-flow and the Tri-flow catheters; and found to cause varying extent of injury. The Aero-flow was superior to the Tri-flow, which was superior to the Bard side-hole, because of the observation that tips designed to minimize mucosal contact were the

least injurious to mucociliary function, presumably by the least interruption and denudement of mucosa.

The cough clearance mechanism provides a rapid, complete exit for particulate aggregations, especially those resulting from the mucociliary pathway. Coughing essentially results from the development of intra-pleural pressure greater than 100 mm of Hg, causing narrowing of the cross-sectional area of large airways with a corresponding increase in transit velocity, as rapid as 85% the speed of sound! In disease entities such as bronchitis, emphysema, or cystic fibrosis, or in intubation, this mechanism is severely impeded (128,42).

Other anatomical points of interest are the pulmonary arteries that branch directly with the airways as far as the respiratory bronchioles, and supply blood to the capillary networks of the terminal respiratory unit. The close structural relationship of the arteries and airways in which collapse of one causes decreased circulation, allows maintenance of a proper ventilation-perfusion balance of the terminal respiratory units (or alveoli).

INTRAVASCULAR FILTRATION AND DETOXIFICATION:

Filtration and detoxification in the lung can also be thought of as occurring on the intravascular side, as well as the endobronchial side as we have discussed.

Although a relatively new concept, ongoing research in this area has begun to provide substantial information about the clearance of drugs and biodegradable substances. Clearance of intravascular microbes by the reticuloendothelial system in the lung is of current interest in several centers; and should be a very rewarding area of investigation.

With a blood vessel surface area of 70 to 80 meters square, the pulmonary capillary bed is the largest in the body; and likewise, unique in receiving the entire cardiac output at any given instant. The lung parenchyma utilizes only 1 to 2% of the total oxygen requirement of the body in order to maintain itself. Although the normal intravascular blood volume of 60 ml. transits the lung in less than one second, the surface area is so enormous that there is ample opportunity for important filtration and transaction to occur (2,46,128).

DRUG REGULATION:

In most conditions and in the normal portal system absorption, the liver acts as the primary site of detoxification of lipid soluble compounds to inactive excretable water soluble catabolites. But there also exists a gut-liver-lung axis, known by garlic eaters for years, by which the gastro-intestinally absorbed agents as well as the hormone gastrin are inactivated (46). Normally, the liver protects the lungs from the portal system contents with as much as 90% of these compounds metabolized in that organ.

The liver and the lung have a similar detoxification system organized into 6 components involving various cytochromes (2,168). Both systems are microsomally located, capable of induction, function non-specifically and have similar protein tissue substrate concentrations activities. However, there are some kinetic differences. The hepatic system is induced by a wide variety of drugs, such as, phenobarbital, while the pulmonary enzymes are primarily induced by carcinogenic polycyclic hydrocarbons found in large amounts in cigarettes. The polycyclic hydrocarbons inhaled in cigarette smoke may induce conversion by the pulmonary mixed oxidase system to reactive epoxides which in itself may be inadequately cleared after reaching a critical level and are therefore felt to be carcinogenic also. The lung has a glucuronal transferase system only 2 to 3% that of the hepatic system, but its role in detoxification is only beginning to be understood. In diseases of the liver such as cirrhosis, metastatic disease or when new previously "unseen" drugs are poorly metabolized in the hepatic microsomal system, the lung loses this protection.

With the introduction of each new ingestible drug, the microsomal conversion system at the liver level is tested as these are absorbed from the portal system. New or unrecognized substances may not be effectively detoxified within the liver system which has not developed sufficient machinery; and therefore, a large overflow will be seen within the lung. One drug which has been increasingly identified with the induction of pulmonary fibrosis in humans is that of bleomycin.

This is dealt with inPhan's paper (136) in which he demonstrated the production of pulmonary fibrosis in rats after only 2 weeks of administration of bleomycin with a concomitant increase in the rate of collagen synthesis being noted. No doubt with an increased awareness of the problem, more information in this area will be forthcoming.

HORMONE CAPACITOR:

The lung is capable of regulating the arterial level of locally active hormones; acting as a capacitor for endogenous and exogenous lipophilic amines, and of acting as an endocrine organ helping to mediate anaphylaxis systemically. Perfused rat lungs rapidly concentrate exogenous lipophilic amines, a list of compounds which is quite extensive and involves such widely used substances as chlorpromazine, propranolol, morphine, tetrahydrocannabinol from marihuana, imipramine, amphetamine, methadone and chlorcyclizine (2). However, except for methadone, these drugs are not significantly metabolized within the lung, but rather may be acutely concentrated, stored and then selectively released over a period of time.

The topic of non-respiratory lung function albeit in its relative infancy is a fascinating one, with the implication that the lung acts as a major homeostatic endocrine organ over and above its primal duty of oxygenation, and recent role of detoxification. The lung distinguishes between local and circulating hormones by selectively filtering and degrading certain hormones while allowing unaffected

transit of structurally very similar compounds. An example of this is the almost complete uptake and degradation of norepinephrine and dopamine in distinction to total indifference to epinephrine vasopressin and histamine which consequently have equal concentrations in both arterial and venous blood (2). Serotonin that may escape degradation in the liver is degraded by about 70% in one passage through the lung (46); and only when massive amounts are produced by a carcinoid tumor, for example, is this double system overwhelmed allowing the well-known symptomatology. The overflow of locally produced hormones bradykinin, SRS, and other vasoactive substances into the general circulation, account for the systemic manifestations of anaphylaxis, which it appears the lung can modulate, as a capacitor. Normally, this only occurs with the proper mechanical and chemical stimulation. Locally active hormones, that is, hormones whose arterial concentrations are determined in the lung, are bradykinin, prostaglandin and angiotensin I. The kinins which are potent vasodilators increasing vascular permeability represent a group of polypeptides that have been associated with pulmonary edema formation, anaphylaxis, and hypovolemic and septic shock. The lungs are exquisitely tuned to degrade as well as generate kinins (46,168). The half-life of bradykinin in the blood is approximately 17 seconds or much less than in one circulation time. Caveolae at the pulmonary capillary endothelial surface is believed to be the site of inactivation by an enzyme which acts as a dipeptidylcarboxypeptidase cleaving the C-terminal dipeptide.

This may, in fact, be the same enzyme as in the hydrolyzation of angiotensin I to angiotensin II, the formation of which occurs when renin released from the kidney acts on the plasma alpha-2 globulin to form decapeptide angiotensin I. This conversion to the active octapeptide II provides an enzyme which is 40 times more potent as a pressor than is norepinephrine. Recently, it has been shown that the conversion of angiotensin I to angiotensin II is depressed significantly by conditions of hypoxia in dogs (168). He showed that the conversion went from a baseline of 72% to that of 6% in one passage through the lung circulation. He and his workers showed likewise a prompt return of the normal conversion level with the return of oxygen tensions.

PROSTAGLANDIN MEDIATION:

Changes in pulmonary blood and air flow can be modulated by nervous, systemic, and local immunologic reactions. Much of the work of mediating these intra and extra-pulmonary changes are prostaglandins. These are a family of polyunsaturated fatty acids, all containing a 20-carbon backbone (prostanoic acid). In effect, the lung is the most important body organ for production and degradation of prostaglandins.

As a general rule, the prostaglandins of the E series are the dilators and the F series constrictors of bronchioles and pulmonary vasculature, with some anomalies. Normally, 60 to 90% of the prostaglandins are inactivated in one pass

through the lung, but this inactivation is impaired experimentally with the administration of endotoxin which radically decreases the inactivation, particularly the PGE-1 which is the potent vasodilator (46,113). Also, PGE-1 appears to have a more prolonged effect than does its counterpart, the PGF-2 alpha. Activation and synthesis is not completely known, but is thought to occur at the microsomal fraction of the tissue homogenate involving 15-hydroxy prostaglandin dehydrogenase, or 15-PGDH, activity as well as an inter-converting enzyme present within the lung tissue. In general, PGF-2 alpha which appears to direct pulmonary blood flow locally is present in high concentrations within the parenchyma and conversely PGE-2 is present in high concentrations within the airways.

Factors which stimulate production of prostaglandins are anaphylaxis which has been studied in vivo in the guinea pig, as well as hypoxia, edema, vitamin-C deficiency and some non-specific stimuli (113). Steroids result in a reduction of prostaglandin production and these may act by decreasing the supply of available substrate from the cell membrane phospholipids by stabilizing the membrane (89).

Prostaglandins may function by their effect on cyclic AMP, particularly the PGE variety which has been shown to increase cyclic AMP. Indomethacin, on the other hand, decreases this

effect in the normal situation, but not in asthma attacks. Aspirin and indomethacin are known to inhibit prostaglandin synthesis. Of importance is the relative balance for providing an equilibrium between the dilatory effect of PGE and the constrictive action of PGFs, the end product being proper tone and perfusion of the airways.

Prostaglandin and thromboxane released from the lung may have variable half-lives, due to variable inactivation by the 15-PGDH (135). Both prostaglandins, particularly the PGF-2 alpha variety and thromboxane have prolonged effects during anaphylaxis. It now appears that the administration of high levels of oxygen for prolonged periods of time (72 hours) can significantly inhibit the function of 15-PGDH. This was examined in guinea pig lungs by Crutchley (32), and he found that 83% inhibition took place after 100% 02 for 72 hours. The resultant effect is a relative prolongation of the anaphylactic condition.

Hsueh (89) and his group have demonstrated that both peritoneal and alveolar macrophages are capable under stimulation of prostaglandin synthesis. Mouse peritoneal macrophages produced PGE-2 over a 90 minute period when stimulated by zymosan. He demonstrated that the addition of indomethacin completely blocks their production. Utilizing zymosan-stimulated rabbit alveolar macrophages, he demonstrated the production of a mixture of prostaglandins, PGE-2, D-2, F-2 alpha and 6-keto-

F-1 alpha. The addition of cytochalasin-B in conjunction with zymosan further increased the total prostaglandin production, possibly by activation of phospholipase, but by itself did not have an effect on prostaglandin synthesis.

PULMONARY IMMUNOLOGY

INTRODUCTION:

> Most meningococci have the sense to stay out on the surface, in the rhinopharynx. During epidemics, this is where they are to be found in the majority of the host population, and it generally goes well. It is only in the unaccountable minority, the "cases" that the line is crossed, and then there is the devil to pay on both sides, but most of all for the meningococci. (172)

In minutes after invasion, bacterium which has penetrated the filtration mechanisms and reached the alveoli, triggers off an appropriate, sometimes inappropriate, immunologic response. This response is sensitive to the type of bacteria, the soluble mediators secreted by and for the participants, and the overall state of health. The goal of the whole process is to incapacitate the organisms to prevent them from becoming pathogenic, and then to remove them.

Lung clearance or a reflection of the percentage microbes remaining after instillation over a period of time is dependant on many factors as pointed out earlier. They are: phagocytosis by the macrophage which requires adequate numbers and viability, alveolar bronchial transport mechanisms, the type of organisms, and in vivo solubility, if any, of the particles, cough mechanism, and clearance lymphatics.

The clearance of aerosolized non-soluble, non-toxic substances from the lung, was studied by Kavet and Brain and found to have a biphasic clearance pattern (102). That is, the first phase was rapid and the clearance took place in less than 24 hours and was largely influenced by the mucociliary clearance. The technique utilized sodium iodine scintillation crystals or magnetic dust which provide non-invasive

sensitive magnetometers with measurements. The second phase took place over a 2 to 10 week period or longer, and had even greater involvement of the pulmonary alveolar macrophage ingestion for clearance from the lung.

The technique of measuring intra-pulmonary bacterial clearance or killing is the technique that we employed in our work. It was first used by Laurenzi, Berman, and Kass in 1970 in which inhaled aerosolized bacteria was followed for clearance by serial sacrificing and colony forming unit quantification. Green and Kass later studied the killing and the physical clearance simultaneously using p-32 labeled Staph. Aureus and Proteus Mirabilis (67). They found at 4 hours that 80 to 85% of the radioactivity in the lung was present. However, only 8.7% of the Staph. Aureus and 23.4% of the Proteus Mirabilis remained from the 0 hour level. So they concluded that the disappearance of the colony forming units may better be accounted for by the intra-pulmonary killing rather than the physical removal per se.

The alveolar macrophage represents the first line of intra-pulmonary defense that meets the bacterial invasion and in their work, Green and Kass (67) counted intra and extra-cellular Staph. Aureus bacteria that was ingested by the pulmonary alveolar macrophages. They noted that ingestion always preceded the decrease or diminution of the colony forming units, again implying the intra-pulmonary killing mechanism as primary, and again with the pulmonary alveolar macrophage in the central position.

In minutes after invasion of bacterium, alveolar macrophages are capable

of intercepting their entry in the lung, by responding to as well as synthesizing chemotactic factors elaborated both by the bacterium and the phagocytes. Indeed, a lung lavage from a normal "unstimulated" individual contains: 47% T cells, 19% B cells, and 34% "null" or lining cells (80), reflecting the important surveillance role of the pulmonary alveolar macrophage. As we shall see from our study results, the alveolar macrophage, when stimulated can respond by increasing both numbers and virulence. The ability to make such a response, particularly with presensitization, indicate the alveolar macrophage is unique in straddling both the surveillance and the augmented portions of the immunologic system.

The interaction of the various components of lung immunology, systemic and particularly local, lends further credence to the alveolar macrophage's role as co-ordinator or "conductor" of the local defense and clearance, at the same time as performing its own multivarious functions.

PULMONARY ALVEOLAR MACROPHAGE:

> Lymphocytes, like wasps, are genetically programmed for exploration, but each of them seems to be permitted a different, solitary idea. They roam through the tissues, sensing and monitoring. Since there are so many of them, they can make collective guesses at almost anything antigenic on the surface of the Earth, but they must do their work one notion at a time. They carry specific information in their surface receptors, presented in the form of a question: is there, anywhere out there, my particular molecular configuration? (172)

-Origin and Kinetics:

The macrophages of various organs in the body derive from progenitors in the bone marrow, and this is true of the pulmonary alveolar macrophage,

(henceforth referred to as "AM"). In fact, AM's are end-stage phagocytes derived from two precursor sources - an uncommitted pleuri-potential hematopoietic stem cell capable of differenting into erythroid, granulocytic, or megakaryocytic cell lines; and a committed differentiated precursor. This latter cell line exists intra-pulmonary and can renew itself as well as mature into functional elements (80).

Evidence for this intra-pulmonary source is research in which syngeneic mice were divided into three groups: whole body, pelvis, and chest irradiated (62). The lung lavage from only the whole body radiated mice revealed deficient numbers of alveolar macrophages, in distinction to the other preparations, clearly indicating the replacement potentials of either a bone marrow or intra-pulmonary source.

The development period from bone marrow to lung is about 100 days, as shown by whole body radiation and bone marrow transplantation in opposite sexes. After transplantation, the donor-origin cell, as evidenced by appropriate karyotype, almost completely replaces the AM population by 100 days (80,61). In effect, there is a multi-compartment model to the evolution - bone marrow stem cell, to circulating blood monocyte, to interstitial cell, and finally to AM. It appears that the AM develops its unique metabolic characteristics during the interstitial phase.

The pulmonary alveolar macrophage is unique among mononuclear phagocytes with differences that largely result from dwelling at the air-tissue interface. This location results in their direct exposure to inhaled micro-organisms as well as toxins including cigarette smoke and air

pollutants, therefore, functioning as the primary defense system in the lung immunology. The aerobic environment has particularly led to metabolic characteristics unique to the AM, and these will be discussed in the section on "Phagocytosis", as the two are intrinsically related. Suffice it to point out at this time, that the AM is uniquely capable of utilizing both aerobic and anaerobic metabolic pathways (167). (See Figure XXXII).

-Morphology:

Alveolar macrophages from the various mammalian species are structurally fairly similar, the human AM is no exception in this regard. Through light microscopy, the human AM has a diameter that ranges from 10-50 microns, with a mean of 25 microns, the cells contain irregular nuclei, large amounts of cytoplasm, with a nucleus to cytoplasm ratio variable but commonly 1:3, and very prominent vacuoles. The cytoplasmic inclusion bodies stain for acid phosphatase and this property was used to better define the lysosomal contents of the AM (62). (See Figure XX).

Although the ultrastructure of the AM varies somewhat according to the phase of cellular activity, electronmicroscopy generally shows frequent nucleoli, as well as cytoplasmic organelles, such as well-developed Golgi apparati, mitochondria, and endoplasmic reticulum of the rough type. The most striking ultrastructural feature of the AM is an abundance of cytoplasmic inclusions having an electron-dense matrix, and correspond to the areas of acid phosphatase stain. These inclusions are found to contain cathepsins, lysozymes, B-Glucaronidases, B-galactosidases, aryl-sulfatase, acid ribonulease, and phospholipases - all associated with lysosomal function, and all in greater concentration

than the peritoneal macrophage. Peripherally, pseudopodia are prominent, and in this case presumably about to engulf a pneumococcal bacterium (62,80). (See Figure XX)

The alveolar macrophage surface contains receptors for the crystallizable fragment, that is, the FC component of IgG and the third component of complement. These FC receptors appear to be important for particle attachment and ingestion by the AMs; and can increase in number as well as size under in vitro stimulation by Freund's Adjuvant for example (80). Rabbits treated with BCG produced AMs with more complement and IGG receptors and with an increased receptor affinity. These changes are felt to correlate with the enhanced capability of macrophages to ingest opsonized micro-organisms.

-Local Replication:

In vitro proliferative capacity is functionally very important for replenishment of the AM, in the steady state; and possibly even more important when under stress. An extensive study of kinetics was reported by van Ord Alblas and colleagues studying AMs in mice after injection of tritiated thymidine (13). The derived labeling index is used as a measurement of DNA synthesis and proliferation by the AM in this case. The highest value in the labeling index in both the AM and the blood monocyte population coincided at approximately 60 hours. Another group was compared after total body irradiation with hindlimb bone marrow shielding. In this case, the number of AMs went up in the first 24 hours and only gradually fell, while the blood monocytes number fell rapidly. These findings indicate a local production of the AM under stimulation, but not in the steady state.

The percentage of locally produced macrophages is estimated at around 10%, and may therefore be significant only under severely stressed and compromised conditions.

Finally, Alblas had calculated the numbers in the turn-over process in AM kinetics, showing that 15% of the blood monocytes that leave the circulation become AMs. Through labeling methods, he discovered an influx of 1.43×10^5 cells into the lungs in a 48 hour period, resulting in a mean turn-over time of 27 days for AMs (13).

Local, as well as in vitro, replication of AMs has been studied further using cloning methods for macrophage granulocyte progenitor cells in a semi-solid condition medium (14). Intra-luminal alveolar macrophages were found to proliferate in both steady state conditions as well as in periods of pulmonary stress. In this study, rat lungs exposed to ozone showed a 600% increase in the colony formation of AMs over the controls, which was noted by day 1 after the insult, with a decline to the control level by 7 days. This time period is similar to that in Alblas' work with the largest increase demonstrated in vivo local proliferation under steady state conditions. Therefore, local proliferation is almost probably only important in the stressed and compromised individual as has been pointed out.

-Retrieval:

In 1961, it first became practical to study the AM after Myrvik described a technique to remove rabbit AMs by lung washings (80). Prior to this and even after, the ease of retrieval from the peritoneal cavity of small laboratory animals provided emphasis on the study of peritoneal

macrophages. The ability to obtain a large enough AM sample by endobronchial lavage has been enhanced largely by laboratory technique.

In man, the ability to obtain and study AMs, essentially the only human tissue macrophage readily available for study, has paralleled the development and use of the fiberoptic bronchoscope. Normally, the bronchoscope is wedged into the right lower lobe bronchus, and the segment washed with 300 ml of normal saline in 50 ml aliquots (60, 77) Usually half is retrieved, and the remainder is readily absorbed. Complications that can occur in the human are those of any bronchoscopy, vocal cord damage, atelectasis, pneumonitis, and fever - all at a low incidence.

Only recently, is it learned that cationic local anaesthetics, the two most common being lidocaine and tetracaine, often used in awake bronchoscopies, have profound, longterm effects on AM in vitro function (83). The authors found that these anaesthetics caused a decrease in oxygen consumption by as much as 67 to 74%, and they also demonstrated, through scanning electronmicroscopy, a marked alteration of the surface membranes of the cells so treated. This information has led, as in the small animal retrieval, to washing the cells well to rid them of the effects.

Utilizing a physiologic response of AMs, that is, adherence to surfaces of tubes and containers on contact, a technique for achieving high yields in laboratory animals, such as guinea pigs, mice, and rats has gained recent popularity. The adherent cells, which would otherwise

be precluded from further studies, are washed in lignocaine, causing loss of adherence, and thereby retrieved. The cells are then washed in lignocaine-free culture medium and placed in non-adhering plastic containers. The author found no longterm effects on in vitro function studies (85).

Another method to obtain a purified AM sample from humans utilizes cell profile diameters (36). With the knowledge that macrophages recently recruited from the pool of blood monocytes into the lung, are somewhat smaller than longer-term lung residents, one group of investigators was able to obtain individual samples of free lung cells with unbiased cell distribution. This was done simply by examining cell pellicles obtained by bronchopulmonary lavage under light microscopy and measuring a cell profile diameter. Arensen used volumetric analysis of blood monocytes to demonstrate a morphologic heterogeneity; for which he was able to make functional correlates (4).

-Monocyte Heterogeneity:

Clearly, all monocytes are not the same, with blood monocytes and macrophages, peritoneal, and alveolar macrophages deriving differences from functions and organ specificities. Meuret studied the above human cell types characterizing their proliferative indices by tritiated thymidine labeling in vitro (116). He also determined bacteriostatic capability by the macrophage's ability to block DNA synthesis of proliferating E Coli after phagocytosis. In most cases, the proliferative activity of these blood monocytes and macrophages from pleural effusions and ascites was less than 1%. However,

macrophages from patients with neoplastic diseases exhibited higher indices ranging between 4 and 9.6%; specifically in cases of breast carcinoma, ovarian carcinoma, seminoma, and lung cancer in descending order. The stimulation, he felt, was secondary to lymphokines.

In contrast, alveolar macrophages from these cancer patients had totally lost their proliferative potential while at the same time maintaining a higher bacteriostatic potential, a fact he ascribes to the relatively more sophisticated alveolar macrophages. One would be hard-pressed to explain the difference on the AM's unique dependance on oxygen as levels of 25 mm of Hg tension, felt to be adequate for normal function, were maintained.

Work with the skin macrophage, particularly by the Chinese, has aided in the development of techniques to demonstrate pulmonary alveolar macrophage replication as well as the tissue and peritoneal type. Using the method of skin blister formation by intra-dermal cantharidin injection, a group from Peking obtains large quantities of macrophages from the blister for study (25). The incorporation of tritiated thymidine has disproven the earlier concept that macrophages of all types were terminal cells in the G-zero phase and capable of little, if any, incorporation. Recent literature including this study indicates that peritoneal, tissue, and alveolar cells demonstrate cellular replication and DNA synthesis as reflected by thymidine incorporation (25,62).

Hocking and Gold showed this replication ability in the recent AM in vivo and vitro, using the radioautographic techniques of tritiated

thymidine labeling (14). They showed that in patients with acute leukemia under intensive chemotherapy, the alveolar macrophage uptake of thymidine was greatly increased presumably to compensate for periods of prolonged monocytopenia from bone marrow suppression.

A unique sub-population of macrophages from mouse spleen and bone marrow has been identified by examining complement receptors (148). It was found that by utilizing the binding and phagocytosis measurements of coated (IgM) and uncoated sheep erythrocytes, the process was facilitated by coating with both sub-populations. This description of complement receptors for the two cell groups is further reflection of the heterogeneous nature and capability of the macrophage.

-Pulmonary Alveolar Proteinosis:

The interaction of surfactant with AMs is a close one that can be both helpful by creation of a microbicidal environment (see phagocytosis), or detrimental by the over-production of surfactant, the principle ingredient of which is dipalmitoyl lecithin. Now thought to be produced by the Type 2 alveolar cell, surfactant can be over-produced or inadequately cleared causing severe depression of antimicrobial capabilities of the AM. This effect is separate from the ventilatory benefits of surfactant which appear to play a role in the etiology of neonatal respiratory distress syndrome, oxygen toxicity, atelectasis, and radiation pneumonitis (75).

In 1958, Rosen first described a disease state from over-production or inadequate clearance of surfactant, and called it pulmonary alveolar proteinosis as the air spaces were filled with lipoproteinaceous

material (60, 96). Patients suffering from this condition have a much greater incidence of complicated pulmonary infections than normal individuals, particularly the exotic varieties, with Nocardiosis the most common.

Gold feels that over-production by the pneumocyte is the primary event in the pathogenesis with the secondary a functional defect in the AMs attendant to engulfment of large amounts of lipid material. On electronmicroscopy, the macrophages contain the same type of lamellar material as surfactant with a marked depletion of lysosomal enzymes (60).

In vitro testing on macrophages from patients with this disease, demonstrate depressed bactericidal function. Utilizing Staph. Aureus organisms in culture, it was discovered that phagocytic capability of the AM was significantly decreased at 2 and 3 hours post-exposure, as reflected by increases in the viable organisms (75). The defect did not appear to be intrinsic to the AM which functions normally immediately after entering the alveolus, but a "glutting" on the surfactant which inhibits phagocytosis of foreign bodies. In Gold's work, the AMs in this condition demonstrated defective adherence to glass, defective chemotaxis, and no defect in phagocytosis in the first hour. It should be noted that blood monocytes in these patients show none of the defects of the AM, as expected (60).

One extrapolation of this conceptual defect on AMs is in cases of pulmonary edema secondary to the leaky capillaries or low pressure type. It is known that pulmonary edema causes depressed bacterial

clearance from the lung (see section on pulmonary edema) and one possible mechanism is the over-ingestion of extravasated proteins by the AM. This may be particularly important in cases of septic shock, burn and/or chemical pulmonary damage, in which protein losses can be very great. I hasten to add this is only an hypothesis at this time; and we intend to pursue this in the clinical situation by examining AM's function and ultrastructure.

The treatment of patients with pulmonary alveolar proteinosis is, in fact, bronchopulmonary lavage and removal of as much lipid material as possible. One ancillary benefit is an adequate supply of AMs for study in this condition. The technique has been used for about 10 years with very good success - both temporary and longterm. A double-lumen endobronchial catheter isolates the two lungs for anaesthesia, with ventilation and oxygenation through one channel and lavage through the other using buffered normal saline at body temperature. After ventilation with 100% 02 for approximately 10 minutes, the denitrogenized lung may then be filled by saline solution equal to its complete FRC as the 02 is absorbed. Lavage can be repeated equal to TV to a total of 20 liters, removing lipid-rich material in the effluent. This technique, used largely by Gold, has been modified for use with asthmatics who may have inspissated plugs with a reduction in the amount of lavage fluid used at any given session limited to approximately 500 ml (77).

-Tumor Cytotoxicity:

The role that the AM may have in anti-tumor immunity is in the process of definition, and certainly is at a very early stage. It has been shown that AMs from mice pretreated with the immunoadjuvant Coryne Bacterium

Parvum are cytotoxic for syngeneic tumor cells (80). This finding may have implications for the evolution of neoplasms, certainly those arising in the respiratory tract.

More extensive work has been done with the tissue macrophage particularly by the Peking group. (25). They obtained T cells for study by blister formation using cantharidin, a cousin to so-called Spanish Fly; and did so in patients with confirmed neoplasms of which there were 48 cases including 33 of esophageal carcinoma. There was a non-specific cytotoxic reaction with two human malignant cell lines with macrophages taken from these patients. They then attempted to alter the clinical courses of patients with squamous cell ca of the tongue by weekly local, peri-tumor injections of this blister fluid containing high levels of T cells. After several weeks of treatment, they did not specify, the tumor was excised and compared with tumor biopsies before instituting injections. In summary, tumor cell degeneration and invasion by giant cells was noted; as well, in one case, complete regression of tumor was reported.

The ability to manipulate this cytotoxic response is shown by using lipopolysaccharide (LPS), a portion of the endotoxin, to stimulate human macrophages from blood monocytes (23). Two adherent human tumor cell lines, adenocarcinoma of the breast and of the colon, kept in culture, were exposed to LPS-stimulated macrophages which were strongly cytotoxic. To test the stimulation, polymyxin, known to from a stable molecular complex with the lipid-A region of LPS,

abolished the stimulated cytotoxicity when added. Clinical use of BCG in melanoma therapy, although not yet clearly efficacious, presumably functions by the same mechanism, as a non-specific stimulator. The phrase "non-specific stimulator" probably belies a rather more complex process involving antibodies, blocking anti-tumors, and T-helper cells which will be discussed in the section on interaction-orchestration.

-Antibiotic uptake:

With greater understanding of the macrophage system, there is an interest in the antibiotic uptake by alveolar macrophages. This may be clinically important with organisms which are capable of intra-cellular survival and they are not uncommon. They include not only the Pasteurella that we utilized in our study, but also Vaccinia, Mycobacterium, Brucella, Salmonella, Listeria monocytogenes, Histoplasma, Candida, Cryptococcus, Blastomyces, Toxoplasma, Plasmodium, Trypanosoma and Leishmania. They all have various techniques to escape from the phago-lysozome. Vaccinia virus escapes after it is uncoated by the lysosomal enzymes. Trypanosomes escape from the vacuole and replicate in the extra-vacuolar space. Chlamydia, Mycobacterium, and Toxoplasma modify the vacuole and block fusion with the lysosomal granules, thereby avoiding digestive enzymes (88). In any case, although antibiotics may not directly pertain to all of these, it is certainly of interest and pertains to the patient who is on longterm antibiotics, such as for chronic bronchitis.

The uptake of 14 commonly used antibiotics was examined by

radio-labeling technique in rabbit alveolar macrophages (98). It was found that the uptake of clindamycin was the greatest and the most rapid with a cellular to extracellular level equal to 50 by 30 minutes. Ethambutal with a ratio of 7; and 2 erythromycin preparations with ratios greater than 20 were all markedly accumulated by the macrophages. Ethambutal, erythromycin and clindamycin uptakes by the macrophages were shown to be dependant upon the oxidative metabolic processes just as any other phagocytic ingestion process.

These findings and certainly more to come will help to guide us in the proper utilization of antibiotics in patients who have severe acute or chronic disease states. These include patients with chronic bronchitis with COPD, patients who have been subjected to thermal injuries, who are on immunosuppressive drugs, who have longterm debilitating diseases, who have been subjected to lengthy anaesthetics and operations, and finally, the patient who is nutritionally deficient.

Meakins has shown that the nutritionally and debilitated patient has good correlation with an anergic cellular response. This state can often be reversed with proper administration of hyper-alimentation (27). This depressed CMI has also been shown in the patients subjected and suffering from Kwashiokor with profound protein-calorie malnutrition.

Antiobiotics are indeed important, but they will never cure

absolutely - only the host defenses are capable of that. They can, however, be used effectively and judiciously to hold the number of bacteria down until such time as the host defenses can respond effectively.

-Exit Pathways:

It is not known which specific route, if indeed any one in particular, is used to remove the "spent" AMs; after either phagocytosis or cell death. Current feeling appears to lean toward the mucociliary clearance. However, what other pathways are available when this clearance is obstructed by an endo-tracheal tube, injured by the toxins that we have discussed, or severely tampered with as in our study? The effect may be particularly important if intra-cellular killing is depressed or defective and viable microbes are not removed from the lung. We examined this problem in our study and model design.

Alblas' radioautographic work in mice showed that most of the macrophages left the alveoli in the airways via the mucociliary pathway. However, he also speculated that a significant number may leave the interstitium by way of the lymphatic channels and travel locally to the bronchus associated lymphoid tissue (such as seen in Figure XXXII which is from our pig studies). From Balt, the macrophages may exit via blood vessels or enter the airways at the level of the terminal bronchi even earlier. The study was unable to provide any additional evidence to support these speculations.

He did however conclude that there are no significant AM sub-populations that have unique or differing kinetics and therefore, entrance/exit pathways (13).

THE ORCHESTRA:

> When the connection is made, and a particular lymphocyte with a particular receptor is brought into the presence of the particular antigen, one of the greatest small spectacles in nature occurs. The cell enlarges, begins making new DNA at a great rate, and turns into what is termed, appropriately a blast. It then begins dividing, replicating itself into a new colony of identical cells, all labeled with the same receptor, primed with the same question. The new cluster is a memory, nothing less (172).

The creation of this memory, in the form of antibodies, involves co-ordinated interaction between the various members of the immunologic "orchestra". This is composed of polymorphonuclear cells, opsonins, and complement factors, and co-ordinated by messages in the form of soluble mediators. Occupying a key position is the AM for first line pulmonary defense, and in large part conducting the proper interdigitation of the orchestral components to produce the memory - antibodies.

-Soluble Mediators:

Much of the interaction between the two effector arms of the immunologic system is co-ordinated by soluble mediators produced by one or the other cell, and essential for proper processing of antigens as well as pathogens.

Moore and Myrvik demonstrated that antigen stimulated T lymphocytes, particularly under aerosol route, release soluble mediators or lymphokines into the local lung tissue. The normal resident alveolar macrophage provides the first line of cell-mediated immunity by responding to these factors.

Analysis of serial specimens of bronchopulmonary lavage fluid, during antigenic stimulation, revealed an increasing cellular content secondary to a polymorphonuclear leukocyte influx (80). This led to the eventual discovery of two components with chemotactic activity. The larger (molecular weight 15,000 Daltons) is chemotactic for both mononuclear and polymorphonuclear cells, and it is felt that this is the activated portion of the fifth component of complement or C5a. The smaller factor (less than 5,000 Daltons) is chemotactic for only polymorphonuclear cells (92). Using stimulated and unstimulated guinea pig AMs, Hunninghake demonstrated that this neutrophil chemotactic factor was produced by the AM in vitro and in vivo. He showed that stimulation by Staph. Aureus, zymosan, and IGG immune complexes induced factor production. This was via the alternative complement pathway as a result of the AM responding through association and fixation of the C3b to the particle surface (51,92). The factor was characterized more specifically as measuring from 400 to 600 Daltons, and as being antigenically and physically distinct from C5a.

It is now known that the human AM can produce at least two neutrophil chemotactic factors, one being C5a. Using cell culture techniques and agarose wells to quantify, a dose-response relationship has been demonstrated between the chemotactic activity of the factor and the number of monocytic cells in the well culture itself (115,175). PHA, con-A, PWM and sepharose protein A all failed to activate mononuclear cells to produce this factor. Rather, the factor could be produced through the stimulation of lipopolysaccharide or the anti-beta-2 microglobulin serum, as well as BCG, zymosan and endotoxin (contains lipopolysaccharide) (122).

These substances also activate the human AM to produce a glycoprotein that is a colony-stimulating substance, capable of inducing stem cell replication of both granulocyte and monocyte series (39). This would imply a rather significant distant control of lung cellular content by the secreting AM.

Discovery of increasing numbers and specificities of these soluble factors, has forced retirement of the general term "lymphocyte activating factor", because factors may in fact act on different target populations. To date, factors that are thought produced by the macrophage are: genetically related factor (GRF), thymocyte mitogenic protein (TMP), T-cell activating factor (TAF), thymocyte differentiating factor (TDF), and B-cell activating factor (BAF) (39). These elaborated factors focus attention on the co-ordinating

activities of the macrophage. Phagocytosis by the AM is an important stimulant of factor release, as shown by a recent study on rats (138). It was the phagocytosis of the Staph. Aureus and not the enterotoxin or non-specific stimulation which caused the appropriate stimulation.

This year, for the first time, it was shown that human AM can produce a chemotactic factor selective for T-lymphocytes (91). The lung AM from patients with sarcoidosis secreted more monocyte chemotactic factor than did blood T-lymphocytes from the same patients. The accumulation of the monocytes in the formation of lung granulomas may be mediated by the local production of this chemotactic factor.

Finally, during viral infections of the lung, the AM may be an important defense, by virtue of its factor secretions. In the rabbit, recent work has found that pulmonary viral infections cause the AM to produce interferon (1). While being non-specific, it is known to be an important anti-viral substance.

-Polymorphonuclear Cells:

The pulmonary alveolar macrophage albeit central, is not the only phagocytic cell involved with lung defense. The polymorph or PMN is capable of phagocytosis, and is certainly prominent in host defense throughout the rest of the body. Any deficit in either poly or neutrophil leads to a marked

susceptibility to infection.

PMNs are also produced in the bone marrow, but have a much greater turnover rate than the macrophage with a half-life of approximately 6-7 hours. In fact, an estimated 120 billion are normally produced each day - equal to the number of erythrocytes produced. In response to the characteristics of the microbial invader, particularly its cell wall, the numbers of PMNs in the lung may vary widely. As we noted earlier the PMNs are normally less than half the number of the AMs on a normal lung lavage (94).

The uptake and phagocytosis of bacteria by the PMN and the AM is facilitated by opsonins. Fusion is completed by binding the FAB receptor to the antibody and the FC receptor to the phagocytic cell.

-Opsonins and Complement:

Simultaneous with chemotaxis, serum opsonins attach themselves to bacterial surfaces and help to phagocytize the micro-organism. The need for opsonic aid varies with bacterial type, particularly the cell wall.

The best characterized are antibody and complement, but other serum proteins may also have this function. Of antibody classes, only IgG is opsonically active and IG-1 and IG-3 are the sub-classes that participate. For

participation, the FAB and FC fragments, that is, the intact molecule is required.

The complement system, part of the humoral arm, is activated by the antigen-antibody complexes in the so called classic pathway forming the chemotactic peptides C3a, C5a. However, C3 itself may be activated by bacterial or fungal mucopolysaccharide, or by elaborated proteases in the alternative pathway, bypassing the C1, C2, and C4 positions. Of import to our study, the alternative pathway, the classic pathway and all the components of complement are required for the full bactericidal properties of serum against gram negative bacteria. Also, activation of the classic or alternative pathway through C3 is essential for the enhancement of phagocytosis in smooth pneumococci. (43, 94,106,118)

The problem of complement absence is cause for recurrent pneumonias, as it is vital for adequate phagocytosis of certain types of bacteria. A clinical study involving 23 pediatric patients with recurrent pneumonias revealed a deficit in the complement chain at the select absence of C2, shown by chemiluminescence and immunoelectrophoresis.(141) The chemiluminescence technique briefly is from a by-product of opsonized bacteria adequately phagocytized with the production of highly reactive oxygen intermediates. These intermediates, such as superoxide anion, chemiluminesce on scintillation counting. When the sera from the patient with the absent C2 is exposed to Staph. Aureus and to E Coli in the absence of any serum replacement, a

defective killing of the Staph. Aureus by the polymorphonuclear cells was shown. Therefore, C2 was identified as an integral component for the adequate bactericidal control of Staph. Aureus, but not of E Coli.

-Microbial Interaction:

Both humoral as well as cell mediated immunity are constantly under the synergistic or inhibitory influence of various microbes and their by-products, which correspondingly, may prevent or promote invasion by a secondary pathogen.

Alterations of humoral immunity can be brought about by organisms which inhibit the production of antibodies directly or indirectly, stimulate production of sensitizing antibodies, or induce blocking antibodies. Known agents for this are the influenza virus in the mouse with the blocking antigen provided by either Strep Pneumoniae or endotoxin; while in the rabbit Neisseria Meningitides stimulates blocking antibodies (109). Also in the mouse, non-specific stimulation of interferon production by erythrocytes, Con-A, phytohemmaglutinin, and lipopolysaccharide causes a suppression in the humoral system (73). The mechanism for this suppression is not understood at present, but several theories are infection of B-lymphocytes themselves, or competitive inhibition of the host's non-committed antibody producing cells by the microbe itself.

The concept of blocking antibodies may have very significant clinical as well as experimental implications, particularly with respect to proper vaccination. For example, live attenuated polio virus given enterally may not produce effective levels of antibodies because a pre-existing enteral viral infection may serve to block antibody formation. The Third World pediatric population is a carrier of many common enteroviruses and therefore, at high risk for failure of proper immunization, with devastating consequences. This phenomenon also explains why some patients with chronic bronchitis or other low-grade respiratory infections may not respond properly to aerosolized pneumococcal vaccine. The common form of this vaccine is called "Pneumovax" and administration is intravenously.

Work with immune complex pneumonitis has revealed that blockade of peripheral lymphocytes can prevent the disease, another example of the interdependance of the two systems. Shanker demonstrated induction of immune complex pneumonitis in rabbits given Con-A, through polyclonal activation of T-lymphocytes in the lung (153). The resultant necrotizing destruction of the pulmonary parenchyma was prevented in rabbits pretreated with intravenous cholera toxin. The toxin effect was to raise levels of CAMP in peripheral lymphocytes and therefore, inhibiting their in vitro proliferative response to Con-A. A further ramification of this study was to decomplement

the animals with purified cobra venom factor, which likewise prevented the immune complex injury.

The mechanism by which microbes impair cellular immunty is thought to occur in a number of ways. One important concept is that micro-organisms alter surface receptors of either macrophages or lymphocytes so that they fail to respond to antigens as well as lymphokines and other chemotactic factors. CMI may likewise be suppressed indirectly by microbe secretion of mediator substances, such as interferon in the case of viral infections which may act as a block at the glycolytic pathway. In patients with secondary syphilis, a circulating inhibitor chalone has been identified which acts to depress CMI. Finally, patients with a marked leukocytosis have a relatively greater incidence of anergy, suggesting that the leukocyte may mediate a suppressor agent as well. The latter situation has been observed co-existent with infections of herpes labialis and other pyogenic infections (100,109).

Clinical studies which help to document these inhibitory relationships is early work from Harvard and N.Y.U. (9,73). In the first, influenza patients were found to be susceptible to secondary pyogenic pneumonias. In the study from Bellevue, the records of 97 TB patients were examined, between the years 1930 and 1941. During this time, 8 definite and 10 probable cases of reactivation of latent TB or exaccerbation of the disease process developed as a consequence of pyogenic

pneumonia. The most common isolated pathogen was pneumococcus.

Experimental work in the area of pulmonary infections has disputed the earlier concepts from the 1950's, that it was the induction of pulmonary edema and cellular debris from the primary infection that provided a suitable culture medium for a secondary infection to take hold. Jacob and Green recently examined the effects on bacterial clearance of pre-infecting mouse lungs with Sendai Virus (95). They measured the rate of clearance of inhaled staphylcocci from both consolidated and non-consolidated lung areas, and reported finding no difference, indicating no effect by local viral infection. However, examination of their data indicated bacterial multiplication only took place in the consolidated areas, which they interpreted as "bacterial migration" to the areas. A more correct conclusion might be that the infection influenced mechanical clearance faculties and/or local T and B cell function.

Correspondingly, there are numerous instances experimentally and clinically, where interactions enhance CMI. An increased resistance to Herpes Simplex virus follows BCG stimulation of macrophages in rabbits; and similarly, Coryne Bacterium Parvum inoculation stimulates cell mediated anti-tumor cytotoxicity in mice as we saw. Clinical application of

this CMI enhancement is the use of BCG scarification in patients with certain levels of malignant melanoma (84,109).

-Antibody Formation:

The need for adequate numbers and function of monocytes is presupposed to aid in the processing of antigen into antibodies both in the lung and in the peripheral circulation. Recent work would seem to confirm this (121). The induced B-cell proliferation to antibody secreting cells was measured in rabbits, after depleting the peripheral blood of mononuclear cells, and stimulating them with Con-A and PHA. Generation of plasma cells as reflected by immunofluorescence and specific-plaque-forming cells, continued virtually unchecked. However, when monocytes were added to the depleted cultures, maximum or optimal response was recorded, indicating an interdependance in immunoglobulin formation.

Although secretory IgA immunoglobulin is in general more prevalent in bronchial lavage fluid than is say IgG, the content and relative amounts are capable of changing according to specific disease states. A question which focuses on an important concept, is do aerosolized antigenic complexes stimulate influx of immunoglobulins into lung by the humoral arm, or are they produced locally? The answer appears to be both. IgG is predominate in peripheral blood, but adequate lung levels are needed to aid opsonization of certain microbes (144). Even in normal subjects the bronchial lavage fluid is relatively enriched compared to peripheral blood,

for both IgA and IgG secreting cells (106). Human bronchopulmonary lavage fluids normally contain IgA, both 11-s and 7-s variety; IgG; somewhat lower levels of IgE; and only occasionally detectable IgM. After administration of antigens systemically, and particularly via the upper respiratory tract, specific IgG and IgA can be detected. Presumably this is as a result of local production by resident B-lymphocytes. The pneumococcal vaccine administered via the airway demonstrates the clinical relevance of this fact (80).

-Immunologic Autonomy:

Local and systemic immunity following antigen deposition in the lung is incompletely understood, but increasingly, studies reveal that the lung responds with localized regional lymph node processing and antibody production, at times quite independant from the rest of the body. It has been shown that after parenteral immunization with Pseudomonas, the IgG agglutinative antibody was present in bronchial secretions which was thought derived from the intravascular pool of IgG by diffusion into the respiratory fluids. In contrast, after intranasal vaccination of Pseudomonas, the antibody activity was detected in both secretory IgA and IgG fractions of bronchial secretions.(143) The immune secretory IgA had an inhibitory effect on the growth of lag-phase Pseudomonas organisms which was specific for the immunizing serotype. In contrast, IgG and the bronchial secretions did not have this inhibitory effect.

Finally, IgM and the complement components were noticeably absent from the bronchial secretions, all of which indicates that the immune milieu of the normal lung is quite different from that of serum.

Selective lymph node drainage in the lung is indicated by recent work with sheep red blood cells which were instilled into the right or left apical and the right or left diaphragmatic lung lobes of 16 dogs (12). The animals were sacrificed 5 days after immunization and suspensions were made from individual lung associated lymph nodes and the spleen, and the results expressed as plaque-forming cells (PFC). The highest levels of PFC corresponded to the lymph nodes on the same side of the lung that received antigen. The response in the spleen was minimal, regardless of the lobe immunized. The number of IgM PFC was consistently higher in the immunized airway than the control with peak responses 7 to 12 days after antigen exposure.

The time period of 7-12 days to process antibodies is commensurate with that quoted in rare work done on the pig respiratory tract. As well, the immunoglobulin distribution in various portions of the respiratory tract, implies a selectivity or specificity of function. In it, the participants examined the upper respiratory tract of the pig and using the immunoperoxidase technique, demonstrated the presence of predominantly IgA, along with IgG and IgM (17). The IgA was first identified at the 6th

to 7th day and was highest at 4 weeks of age with the morphology of plasma cells well developed. IgG was greater than IgM in the lung and in the nasal mucosa, whereas IgM was greater than IgG in the trachea and the bronchus.

Clinical studies examining the non-AM immunology of the lung was performed in two groups of patients - one with idiopatic pulmonary fibrosis (IPF), and the other with chronic hypersensitivity pneumonitis (CHP). Lavage fluid from the lungs of patients with IPF revealed inflammatory and eosinophilic responses and a significant elevation of the IgG fraction. With corticosteroids, the inflammatory cells diminished but the eosinophils remained (144). Fluid from patients with CHP also had eosinophils and elevated levels of IgG, but in contrast contained elevated IgM, fewer inflammatory cells, and a strikingly increased number of lymphocytes over the patients with IPF. Also, the high ratio of T to B lymphocytes found in CHP compared to peripheral blood would again indicate that the T cells may be sequestered in the affected pulmonary areas and pulmonary tissues. Portions of the lung immunology, therefore, appear to function partially independant of the systemic humoral and cell-mediated immune systems (77).

In an extension of the above work, this group made a significant observation about the validity of the lavage fluid analysis.

They compared the bronchoalveolar lavage technique with

open lung biopsy and cell extraction from the biopsy material in 21 symptomatic patients with progressive pulmonary fibrosis (77). In this study, patients were followed by closed lung needle biopsies as well as examination by bronchopulmonary lavage fluid aspirant.

In this study, there was little clinical correlation in the scores of the cell types that were observed in the alveolar spaces and alveolar walls, nor in the differential or total cell count obtained by biopsy. However, there was good correlation between differential counts obtained from lung lavage, so that it would appear that lavage itself more accurately reflects the cellularity of the peripheral portions of lungs not effected by overt bronchial disease. Likewise, steroid responsiveness of the underlying pathology correlated well with the percentage of lymphocytes found in the extraction samples. With steroids, there was an associated fall in the percentage of the macrophages, and none in the numbers of neutrophils or eosinophils. There was, however, a trend towards increased numbers of lymphocytes in the lung washed in those patients who did respond to steroid treatment. In follow-up studies, the cases having predominant lymphocytes in the lung lavage fluid continued to fare well. The other cases with predominant neutrophils or eosinophils showed a less lasting response to the steroids and often deteriorated back to the original pathology.

The relative ease of the lavage technique and the accuracy

of the fluid cellular examination should certainly provide incentive for more utilization in the management of respiratory disease. Especially important is the relatively non-invasive quality versus a needle biopsy, which besides having increased associated morbidity may not be as helpful as the lavage examination.

TOXIC SUBSTANCES:

-Environment:

Environmental and industrial hazards have become the focus of attention in the last 10 years, particularly in the aftermath of such tragedies as the poly vinyl chloride (plastics) workers and Love Canal. Even better recognized is that exposure to asbestos (insulation industry) and to silica (coal miners), can produce localized as well as extensive lung injury. This probably occurs by autolysis of normal tissue with extruded lysosomal enzymes from activated macrophages. That the process can be active and ongoing for years after the last exposure has only recently been learned.

Schulyer and colleagues examined the bronchopulmonary lavage from patients known to have had complicated silicosis, with a last exposure to silica 1-12 years prior (151). The samples were compared with 10 normal subjects who were matched in other respects, all were cigarette smokers. No difference was shown in either the number, or functional studies of the macrophages. However, there was a significantly

increased number of Type-2 pneumocytes present; representing a Type-2 cell hyperplasia. The authors conclude that this is indirect evidence for continued stimulatory presence of a biologically active material years after exposure with presumably adequate phagocytosis by the macrophages.

Other causes of this type of hyperplasia of the granular pneumocyte are such stimuli as oxygen, viruses, nitrogen dioxide, carbon tetrachloride, and radiation (144,151). However, regression is immediate and complete after removal of any of these stimuli. One facet of the disease silicosis, which the authors failed to note, is the obstructive lymphangitis which occurs. This might account for the poor clearance of a biologically active material, in spite of adequate phagocytic level and function.

As is obvious, the distribution of any inhaled substances throughout the respiratory tract depends on the size of the particulate substance as well as on the solubility if a gaseous substance is involved. As we have seen, asbestos, for example, although 300 microns in length, is able to reach the alveoli by virtue of its ability to align itself longitudinally in the airways. On the other hand, water soluble gases, such as sulphur dioxide can be absorbed in the nasopharynx, and in all probability do not reach as far as the alveoli. In contrast, the relatively insoluble gases, such as ozone and nitrogen dioxide may reach the alveoli and thus expose the macrophages to direct toxic effects. Ozone is known to enhance the susceptibility to

respiratory infection by reducing phagocytic bactericidal capability of the macrophages. As well, nitrogen dioxide can cause depression of in vitro macrophage phagocytic antibacterial studies (80,46,128).

Trace elements such as cadmium, nickel, manganese, chromium and vanadium are toxic to the macrophages over prolonged exposure. The injury is shown to be a result of uncoupling of phosphorylation with inhibition of oxygen consumption, so important to the alveolar macrophage (80).

Iron oxide, inherently a non-toxic particle, when inspired is capable of producing extensive damage to the normal lung tissue, through abnormal, non-specific stimulation of the macrophages. Grant showed that in rabbits (66) a 3 hour inhalation of submicron iron oxide was sufficient to cause longterm effects on the macrophages. Lavage revealed their number and quantity of hydrolase enzymes significantly increased.

-Smoke Inhalation:

For the surgical burn patients, respiratory complications are not uncommon, and probably have multiple etiologic factors as we shall see in the discussions on trauma and ARDS. One direct effect of inspired smoke is on the surfactant activity, and this was recently examined in dogs. A standard dose of smoke, wood and kerosene, was delivered at 37°C to mongrel dogs (130). They were monitored for pulmonary and systemic hemodynamics,

respiratory mechanics and surface tension area curves, all as an indication of the surfactant activity. After the smoke exposure, dense, non-segmental atelectasis developed with a fall in Pa02. Surfactant reduction was significant, enough to cause an increase in the minimum surface tension from 7 to 22 dynes per centimeter.

By placing a filter of .1 micron pore size between the smoke source and the endotracheal tube, complete physiologic protection and preservation of surfactant was demonstrated. In understanding the pathophysiology of dense atelectasis, it is felt that loss of adequate surface tension levels provided by the surfactant in alveoli increase the driving pressure required for the capillary cirulation within the lung itself. As a reflection, the pulmonary vascular resistance increases, therefore, the surfactant influences the flow and resistance in alveolar capillaries by changing the surface tension, at the same time as preventing alveoli collapse for ventilation (31,130). Surfactant inactivation, the mechanism of which is as yet unknown, may be one of the first effects of smoke exposure and the consequences of this make the lung vulnerable to additional insults. In particular, the surfactant loss might explain why victims of smoke inhalation are so vulnerable to respiratory complications, and especially so if they have thermal burns as well.

-Cigarette Smoke:

Chronic obstructive pulmonary disease (COPD) is a very debilitating and widespread problem that has developed essentially over the past fifty years - commensurate with the popularization of cigarette smoking. It is now evident that the AM is instrumental in the production of COPD in this circumstance; and is best summarized simply as a stimulation imbalance.

Cigarette smokers, as well as chronically exposed non-smokers to a lesser degree, show similar changes in AM morphology and behaviour. Some of these changes are the AMs become larger and more numerous as shown by broncho-pulmonary lavage differentials (61). The smoker AM is a very activated one with a greater metabolic rate, reflected by an increase in glucose utilization, and hydrogen peroxide production without stimulation of the HMP-shunt.

Ultrastructural changes that take place are that the surface membranes of AMs from smokers are more convoluted possessing more lamellae pseudopodia, as well, there are increased inclusion bodies that characteristically contain kaolinite crystal particles. Transmission EM has also shown these particles in marijuana smokers and non-smokers exposed (167). Hinman used insoluble elastin labeled with tritiated thymidine to show that smokers' AMs secrete 5 times more lysosomal enzymes than did non-smokers (79).

There was not only a consistently higher cell yield in smokers, but a higher percentage of macrophages than lymphocytes which is reversed in the non-smoker.

More specifically, the AM is in a pivotal position between proteolytic enzymes and their inhibitors. Elastase levels are markedly higher in the smoker than non-smoker, and this enzyme group is very important in creating COPD, when not held in check. The anti-proteolytic enzyme, alpha-1-antitrypsin is largely stored in the AM. However, in the smoker AM, this inhibitor is found depleted and rather, a relatively high concentration of proteolytic and elastolytic enzymes collect within the AM. The result of this "unchecked stimulation" is autolysis of normal lung parenchyma, with the acinar and panacinar changes so characteristic of emphysema (167,79,80).

Another recently discovered specific effect of cigarette smoke that predispose smokers to increased rates of pulmonary infection is on the complement system. Patients with COPD have lower C3 and C4 levels than normals as was shown by a group from the Mayo Clinic (118). Serum concentrations of C3, C5 were made on subjects with a forced expiratory volume in one second less than 70% predicted, and compared to matched pairs for age, sex and occupation. Significantly, lower blood levels of C3, C4 were found in COPD patients, and there was a significant correlation between the level and such symptomatology as coughing. The authors could not

differentiate between a depressed production rate or an increased consumption rate causing the low C3, C4 levels.

Airway effects of cigarette smoking can be the severely restrictive - obstructive disease of COPD, or as we now are learning, subclinical but significant abnormalities. Indeed, even non-smokers, chronically exposed to cigarette smoke either at home or in the work environment, show significant airway changes. Twenty-one hundred middle-age non-smoker subjects were studied (181), and "passive smokers", regardless of sex, exhibited lower forced mid and end-expiratory flow rates than did non-smoker controls. Even more significantly, their values were not significantly different from those of light smokers.

For severe COPD, there may be a compensatory mechanism to improve ventilation in the form of collateral ventilation. At this point, collateral ventilation is largely a theoretical concept, although some interesting research in this area is ongoing. The concept is one explanation how occluded alveoli and bronchioles can be supplied with some extra-anatomic oxygenation. In the normal supine individual, the high collateral resistance minimizes collateral air flow through what anatomists call the Pores of Cohn at the alveoli level, and the Ducts of Lambert at the terminal bronchiole. When peripheral airways become obstructed or obliterated in emphysema or other COPD conditions, collateral channels may provide for a more even distribution of ventilation.

The compensatory mechanism represents one more way in which the lung can theoretically attempt to maintain V/Q balance; theoretical because no significant beneficial effects have been shown clinically, and it remains entirely experimental (30,42).

A final "voluntary" toxic inhalant among humans is marijuana. The effect of marijuana inhalation, not THC or tetrahydrocannabinol, is mentioned largely because of the frequent use - roughly 50 million people in North America alone, have been estimated to be sporadic users and a lesser number regular consumers. With a question before courts of legalization of possession of small amounts, this may well escalate. The effect of marijuana has been examined in rats, who were exposed to extracts of purified THC, to the marijuana smoke itself, to a placebo, and a final group were untreated. They then were exposed to aerosolized Staph. Aureus with quantification of intrapulmonary bacterial growth made through the pour-plate technique (90). There was a decrease in antibacterial capability which was dose-dependant, and was effectively a reflection of bacterial clearance. However, in the study, there was no in vitro examination of macrophage or polymorphonuclear functions. The decrease in antibacterial capability was only with association of the whole marijuana smoke and did not come about with animals treated with the THC smoke extract itself.

The theory that has been put forth by Green and his co-

workers (67) suggests that oxidants in the gas phase of the marijuana, as well as tobacco smoke, alter the activity of certain sulfhydryl containing enzymes in the macrophage and depress the energy producing pathways required for phagocytosis. One other consideration that must be kept in mind with respect to the toxic effect of smoke inhalation is the depression of mucociliary response which has been demonstrated certainly with tobacco smoke.

-Gases:

Halothane is not often thought of as a toxic substance capable of depressing antibacterial lung function. That it may be and does is demonstrated in Manawandu's work exposing anaesthetized mice to radiolabeled Staph. Aureus (111). Lung tissue cultures revealed decreasing clearance with increased halothane levels; and particularly decreasing ciliary activity as measured by radioactive washout, with levels of 3% and higher. Commonly, a level of 4 or 5% halothane is used during the induction phase of anaesthesia. The deficit appeared reversible, with a dose-dependant duration requiring up to 48-72 hours. Halothane is known to depress oxidative-phosphorylation which both the macrophage and the cilia utilize for energy, and this might be the basis for the deficits.

Hyperoxia in the neonate is associated with hyaline membrane disease, as is prolonged hyperoxia with a variant in the adult. The effects on mucociliary function have been dis-

cussed, but it now also appears that the alveolar macrophages are adversely affected as well. The interaction of ambient oxygen tensions and macrophage maturation rate, as measured by superoxide dysmutase activity, was examined by Simon and co-workers (157), using mouse alveolar tissue cultures.

In hyperoxic conditions, that is a P02 of approximately 640 mmHg for 24 hours, there was a significant increase in dysmutase activity, reflecting an increased maturation rate over normoxic conditions, where enzyme values were half. Hypoxic conditions with a P02 of about 15 mmHg produced a significant decrease in the dysmutase activity as compared to controls, but only after 168 hours exposure. This delay in measurable injury, might well reflect the compensatory facility of the alveolar macrophage to provide energy, at least for the short-term anaerobically (16).

-Drugs:

Carmustine, or BCNU, used in treatment of malignant gliomas is shown to cause pulmonary toxicity in 20% of users in a recent study (5). Pulmonary interstitial fibrosis is also reported in rats treated with systemic BCNU (8), and both reactions are dose-dependant. Predisposing factors for development are a history of lung disease (particularly ½ pack cigarette smoker a day), length of treatment, and finally, total dose per square meter.

The effect of longterm corticosteroid therapy on monocyte

chemotaxis in humans was examined recently. It showed that macrophages from patients on steroids show a normal random movement and a normal response to chemotaxis or chemotactic factors (169). When prednisolone was added in vitro, these monocytes did not suppress in the same manner as did monocytes or alveolar macrophages taken from 5 normal subjects. He theorized that the depression by as much as 48% of the chemotactic response by the so called normal monocytes was altered in the patients' monocytes on longterm steroids because of a receptor change. He also demonstrated monocytopenia 4 to 6 hours after steroids were given, but with a return to normal at 24 hours. This raises the question as to whether people on longterm steroids, say people with Crohn's disease, as well as patients with sarcoidosis, may reach a plateau effect and get no further benefit from steroid therapy.

-Trauma:

Physical trauma, either surgical, or burns, or extensive or penetrating body injury has had a relatively small amount of study with respect to the effects on the immunologic capabilities of the organs. One person who has worked extensively in this area is Saba who studied the effects of Noble Collip drum trauma on the reticuloendothelial function of rats (100). He showed that both hepatic RE phagocytosis and plasma opsonic activity were depressed by as much as 50% for as long as 6 hours after the trauma. Likewise, the plasma opsonin level declined in direct

relationship to the degree of trauma. Interestingly, he has characterized this opsonin as an alpha-2 globulin designated as a surface binding globulin. He has been able to replenish the RE system when he returns this purified opsonic protein to animals after operation; and even in several cases, he has shown the effectiveness in humans in the form of the alpha-2 opsonic glycoprotein (68,100).

Human blood monocyte cultures can synthesize and secrete plasma protein fibronectin normally concentrated in connective tissues (3). This high molecular weight protein has been shown to promote clearance of micro-particulate fibrin and denatured collagen or gelatin from body fluids. Although the clinical relevance of this work from Finland published this year is not known, its similarity to the glycoprotein, identified by Saba and co-workers is very obvious. Both proteins, both ubiquitous, and both are involved in clean-up rather than immunologic functions per se.

Trinkle and his colleagues examined pulmonary bacterial clearance in dog lungs after reproducible contusion formation (146). His model for contusion is the firing of a 22-cal blank against a quarter which is placed on the chest wall in the same location. Staph. Aureus and Klebsiella Pneumoniae in concentrations of 10^8 and 10^9 respectively were instilled through a Bird Respirator,

in a manner similar to our experiment. Dogs were divided into four groups: contusion alone; contusion plus hemorrhagic shock; contusion plus overhydration with Ringers Lactate; and contusion plus IV corticosteroids in pharmacologic doses. Lung tissue in the areas of contusion and non-contusion was sampled at 4 hours, homogenized and cultured to quantify residual viable bacteria.

Bacterial clearance was not affected by contusion alone. However, any of the three other factors in conjunction with contusion, resulted in significantly depressed bacterial clearance. The study did not provide data on the effect of these manipulators in non-contused areas. Clinical implications are enormous when one considers that one or more of these occur in the insult and ensuing care of the very sick.

-Adult Respiratory Distress Syndrome:

The final common pathway that many disease states manifest and resulting in the clinical and laboratory picture of ARDS, now has some basis in pathophysiology. Entities such as shock, transfusion reaction, hemodialysis, severe trauma, acute pancreatitis, Purtscher's Syndrome, and even myocardial infarction all show pulmonary leukostasis on close examination (94). The mechanism appears to be macrophage stimulation with production of the activated portion of C5 to C5a. Experimentally, C5a has been found to produce ARDS or shock lung in and of itself after prolonged circulation.

One interesting finding is that with increased exposure to C5a, the polymorphonuclear cells become unresponsive to the chemotactic action. This could account for the variant time periods involved in the production of ARDS, as well as variants in severity (21,48,68,70,82).

Macrophages appear to have a central role in this process. In vitro studies involving cultures of endothelial cells from human umbilical veins when exposed to activated complement and granulocytes release toxic oxygen species, superoxide anion and hydrogen peroxide (94). Both are produced by macrophages in normal phagocytic oxidative phosphorylation, and both cause substantial endothelial damage, as shown by the addition of oxygen detoxifying enzymes, superoxide dysmutase and catalase which prevented the damage (68,82).

Complement activation via the alternative pathway also takes place when contact is made with some lipopolysaccharides, importantly the endotoxins; as well as silicone polymers in membrane oxygenators used for extra-corporeal perfusion. Fountain and co-workers (48) demonstrated a non-specific stimulator of the alternative pathway like zymosan to be capable of producing these same effects. In sheep and rabbit preparations so treated, he demonstrated a significant fall in arterial PO2 and a marked rise in pulmonary vascular resistance. More specifically, in hemodialysis, plasma contact with the cellophane membrane of the dialyzer results

in complement activation by the alternative pathway. Laboratory animals in Jacob's work, so treated, have a significant leukopenia with sequestration of neutrophils and monocytes in lung microvasculature, resulting in increased lymph flow as well as respiratory abnormalities. Chronic hemodialysis treatments, although undeniably essential, represent a hazard to the respiratory system by this repeated plugging of the microvasculature.

As an extension of their leukocyte aggregation work, Jacob and colleagues (94), examined the effects of corticosteroids on this action. A dose of 30 ml per kg prevented the in-vitro aggregation of leukocytes, as well as the production of harmful superoxides.

Hemorrhagic shock and the massive fluid infusion (whether colloid or crystalloid) attendant for resuscitation, is commonly associated with acute respiratory failure or the so called "shock lung". Recent evidence shows that injuries sustained at the lung level result not only from the resuscitation period, but from the hemorrhage itself, even relatively small amounts. Connel describes the development in the canine lung of ultrastructural lesions during hemorrhagic shock (31). Platelet and leukocyte aggregation is the earliest changes preceding shock, such that even blood loss too little to cause changes in systemic blood pressure, cause aggregations which occlude microvasculature in the lung. The occlusion is most evident during shock, and the resulting gross

pulmonary hemorrhages and fine structural changes can be demonstrated by electronmicroscopy.

During the study, screen filtration pressure was used as a measure of blood flow pressures through a screen, which is known to accurately reflect the amount of aggregated platelets and leukocytes, as well as debris present in the blood. The formation of lung lesions was time dependant requiring up to one hour for endothelial damage. Further, reinfusion of shed blood following hypotension increased severity of the lung lesions. Reinfusion injury was largely negated if dacron wool filters were used to presumably extract the microemboli.

This observed and proposed pathophysiology correlates well with several other workers the first of whom, Gerdin, demonstrated a markedly delayed clearance of fibrin from the rat lung after acute hemorrhage (57). More specifically, a decrease in fibrinolysis appears to be the main cause of decreased removal of these thrombin induced fibrin deposits. A secondary, but important factor, is inadequate perfusion, at least, to certain areas of the lung.

The so called reperfusion injury which has been described sometimes following shock, pulmonary embolectomy, and prolonged cardiopulmonary bypass (particularly earlier) is examined by Modry and co-workers (119). The study was performed on dogs and focused on bioenergetic, metabolic,

and ultrastructural changes in prolonged total bypass. Ventilated lung tissue was found to tolerate up to 5 hours absence of pulmonary artery perfusion without significant damage. However, 24 hour interruption resulted in significant decrease in ATP and glycogen, and increase in lactate. Reperfusion of these lungs caused even more pronounced biochemical and ultrastructural deterioration as well as frank hemorrhagic pulmonary edema.

The interaction between severe respiratory failure and pulmonary systemic hemodynamics was examined in 30 patients undergoing therapy for failure of diverse causes. (184) With time, the pulmonary vascular resistance progressively decreased in survivors, whereas the converse was true with non-survivors. Three-fold elevations of mean pulmonary artery pressure above normal at equivalent cardiac indices reflected, they felt, a large reduction in the vascular cross sectional area in the lung. Possible causes of this effect are: active vasoconstriction, decreased lung volume, increased interstitial pressure, endothelial cell edema, diffuse microemboli, and finally, microvascular obliteration by fibrosis or extravascular hemorrhage. The author hastened to point out that the numerical results do not define the anatomical position of the vascular reduction, either capillary, arterial, or venous. The common finding in severe respiratory failure of a large ventilation-perfusion abnormality (V/Q) or effective shunt is certainly in keeping

with the proposed pathophysiology.

PHAGOCYTOSIS:

> The phagocytes won't eat the microbes unless the microbes are nicely buttered for them. Well, the patient manufactures the butter for himself alright; but my discovery is that the manufacturer of that butter, which I call opsonin, goes on in the system by ups and downs ... There is at bottom only one genuinely scientific treatment for all diseases, and that is to stimulate the phagocytes.
>
> George Bernard Shaw

The business of alveolar macrophages is phagocytosis and a thorough understanding of that process is necessary as a background. As far back as Metchnikoff, nearly a century ago, this concept of phagocytic cells was put forth and it was with some mysticism that George Bernard Shaw in the Doctor's Dilemma popularized the concept.

Along with the AMs, we know the neutrophils are also involved in phagocytosis, but there are major differences between the two cells. Unlike the polymorphonucleocyte, the macrophage has a much longer generation time, of 19 to 25 hours and a circulation time of 30 to 100 hours, as we have seen from Alblas' studies for example. In distinction to the neutrophil, there is no large reservoir of cells in the bone marrow; but with inflammatory conditions requiring phagocytosis, the production rate increases markedly, mediated by the soluble chemotactic factors that we have discussed. During the maturation from the blood monocyte to the tissue

level, the morphology of the monocyte is altered depending on the tissue to which they are targeted. The AM, therefore, represents a relatively sophisticated phagocytic cell after proliferation from the circulating monocyte.

The process of phagocytosis of necessity involves 4 stages, that is, chemotaxis, opsonization or recognition and attachment, ingestion, and intracellular killing. Chemotaxis, we know, is principally brought about by microbes which activate complement in serum producing C3a and C5a, and the release of mediators. The action of chemotaxis is energy dependant involving actin, myosin and ATPase both in granulocytes as well as monocytes. The recognition of the microbes is facilitated by the opsonins which I have mentioned, IgG and C3 being the most important, and ingestion takes place by a manner of pseudopodia-fusion around the distal side of the particle. This itself is energy dependant, with actin-myosin being identified in the microtubular formation of the pseudopodia. The fusion in turn forms a phagocytic vesicle or a phagosome whose lining is actually from the inverted plasma membrane. In the process of identifying the microbes, opsonins are needed, particularly when defending against encapsulated bacterium, such as Pneumococci, Pseudomonas, and Streptococci (13,61,108,167)

Prior viral infection suppresses intrapulmonary bacterial killing by the AM by causing a defect in the phagolysosome fusion demonstrated by fluorescein microscopy (96). Mice

were given sublethal doses of Sendai Virus, after which progressive inhibition of the phagolysosome fusion was demonstrated reaching a low at 7 days when only 13 +/- 3% of the phagocytic cells fused, as compared to the uninfected controls of 97 +/- 3%. After the 7th day, there was progressive incremental increase in the fusion capability. The author, Jacob, feels that the primary defect is a functional one, as the number of AMs increased 10 to 500-fold during the infection. It is the suppression of fusion that prevents the hydrolytic and lysosomal enzymes from coming into contact with the ingested organism or foreign body itself.

After extensive endocytosis, the phagosomes lose their large surface infoldings, as demonstrated by ultrastructural examinations (see Figure XX) However, the macrophage, in distinction to the neutrophil with its shorter half-life, is capable of replenishing its surface membrane receptors as well as its lysosomal enzymes. The microbicidal process then takes place after thorough ingestion through the pinocytotic release of the lysosomal enzymes in merging the primary and secondary granules with the phagosomes. These vesicles engage in degranulation and microbicidal activity.

This action produces hydrogen peroxide by way of a superoxide anion which in itself may react with already formed hydrogen peroxide to form reactive hydroxyl radicles. This whole process is an extremely rapid one after ingestion.

Because the outer surface of the plasma membrane has become the inner surface of the phagosome, the content of hydrogen peroxide and other intermediates builds up rapidly without injury to the surrounding tissue or organ itself. Hydrogen peroxide is capable of killing bacteria, fungi and viruses by itself, and as well, the myeloperoxidase and halide ions enhance its ability to kill.

The capacity of the organ to detoxify the hydrogen peroxide is provided by catalase which combines to form water, which is one check and balance that is provided for this "lethal machine". Enzymes that degrade superoxide, such as the superoxide dysmutase are contained by certain microbes, in particular, aerobes contain abundant amounts of dysmutase and therefore, are largely insensitive to the superoxide intermediates. However, this dysmutase facilitates the production of the hydrogen peroxide which in itself is even more lethal than the intermediates, so that this is a moot point. The acidity of the phagocytic vacuole, with a pH of 3 to 4.5, possibly secondary to the lactate accumulation, is in itself bacteriostatic at best and bactericidal most probably. The low pH encourages formation of the hydrogen peroxide as well.

In contrast to the 15 to 20-fold increase in PMN oxygen consumption during phagocytosis, the AM undergoes only a

minimal respiratory burst as it were. The AM has the in vitro capability to function both through the oxidative phosphorylation pathway, as well as by the hexose-monophosphate shunt with increased glucose oxidation. The ability of the AM to utilize both pathways is probably a response to the changing oxygenation of all portions of the lung. FigureXXXIII isasummary of these metabolic pathways that importantly produce microbicidal enzymes in the process. The production may be mediated by NADPH oxidase, representing a possible link between respiration and activation of the HMP shunt (80,128,167).

The hexosemonophosphate shunt activity can be demonstrated by the measurement of Carbon-14 labeled glucose conversion to CO2. Under normal aerobic conditions, this HMP shunt activity is only minimally elevated in the AM. Under anaerobic conditions, however, the AM shows in vitro inhibition of phagocytosis, in distinction to the peritoneal macrophages and PMNs in which there is no suppression. Phagocytosis is likewise suppressed in these latter cells when the glycolytic inhibitors iodoacetate and sodium fluoride are added (157). In the rat lung macrophage, aerobic predominance is reflected by the high levels of cytochrome oxidase and low levels of pyruvate kinase enzymes; while conversely, the peritoneal macrophage contains high levels of pyruvate kinase and phosphofructokinase, a reflection of its anaerobic predominance. Interestingly, the AMs when incubated in an anerobic environment, changed

to an enzymatic pattern similar to the peritoneal macrophage, demonstrating a measure of adaptability that is unique (167).

To date, the studies have been sparse in the area of metabolism and ultrastructural changes with no good correlation. As well, most of the literature in this area is from studies on the peritoneal macrophage, for reasons that have been mentioned earlier.

Besides serving a mechanical function in the lung, surfactant is now known to be intimately involved with the bactericidal activity of the alveolar macrophage. In fact, 25 years ago, Macklin proposed just such a relationship between the AM and surfactant on the basis of morphologic observations (80,44). Precursor labeling for the principle component of surfactant, dipalmitoyl lecithin, has established the Type-2 alveolar cell as the primary site for synthesis. The AM, as part of the normal steady state system as well as during activated phagocytosis, is essential for surfactant processing, containing the necessary hydrolytic enzymes to degrade this lipopolysaccharide. Work in the rabbit model has shown in vivo envelopment of the AM by surfactant; and when stimulated the AM utilizes the lipoproteinaceous material to produce hydrogen peroxide, thus creating a bactericidal environment (44).

Lavage fluid obtained from smokers contains more AMs than non-smokers, but significantly less surfactant (see toxic

substances). Whether this fact helps account for the reportedly higher incidence of respiratory infections suffered by cigarette smokers would seem only logical, although as yet unproven. Significantly, it has been shown that atelectasis per se does not result in the decreased production or quantity of pulmonary surfactant (44).

Disorders of monocyte phagocytosis is divided into primary and secondary. The disorders of the primary group can be divided into four functions: production and maintenance; locomotion, chemotaxis, and ingestion; degranulation; and peroxide production (88,167). Disorders in antibody and complement systems deprive the macrophage of much needed opsonins and chemotactic factors, as we have seen.

Listed by function, these disorders are:

Production:	bone marrow dysgenesis, bone marrow ablation secondary to acute leukemia, aplasia, fibrosis.
Locomotion/ Ingestion:	Actin dysfunction, chronic mucocutaneous candidiasis by serum inhibition of chemotaxis, Chediak-Higashi syndrome.
Degranulation:	chronic granulomatous disease with decreased NADH oxidase, 02 consumption, and degranulation; Chediak-Higashi syndrome.

Secondary disorders of the macrophage can be arbitrarily divided into two - Trauma secondary and Disease secondary:

A. Trauma -

Burn/operation:	chemotaxis depressed ingestion increased.
Burn/Trauma:	opsonization depressed
Burn:	Microbicidal depressed
Operation:	RE System depressed

B. Disease States -

Production:	Acquired neutropenias and marrow ablation secondary to toxins, sepsis, drugs; hypersplenism.
Locomotion:	Infections, protein, calorie deficiency, diabetes corticosteroids, rheumatoid arthritis, malignancy, myeloma, uremia, cirrhosis, sarcoidosis, leprosy, Hodgkin's.
Ingestion:	Infection, diabetes, decreased opsonins, and
Peroxide:	None known.

In general, secondary phagocytic disorders are not due to just one defect, but a number that may all be interrelated by malnutrition or preceding illness for example (80,81,88,167,138)

-Bacteria:

> When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockade, seal off, and destroy all the tissues in the area. Leukocytes become more actively phagocytic, release lysosomal enzymes, turn sticky, and aggregate together in dense masses, occluding capillaries and shutting off the blood supply. Complement is switched on at the right point in its sequence to release chemotactic signals, calling in leukocytes from everywhere. (172)

During our work, we examined two bacteria in particular - Streptococcus Pneumoniae and E Coli. Both are relatively strong B-cell stimulators, a behaviour which is related to the structural components of the bacteriae, particularly, polysaccharide.

-Escherichia Coli:

E Coli is an enteric organism that is from the large, heterogeneous group of gram negative, non-sporeforming rods which normally dwell in the intestinal tract. They possess complex lipopolysaccharides in the cell walls which are known as endotoxins. As we have seen, endotoxin can activate the alternative pathway of complement cascades.

The coliforms are classified by a complex antigenic structure - by heat-stable somatic O-antigens, by heat-labile capsular K-antigens, and by flagellar H-antigens. The E Coli are typed according to the K-antigen of the polysaccharides covering the somatic antigen (21,76,97).

Streptococcus Pneumoniae are gram positive diplococci, arranged in chains and with a polysaccharide capsule that permits easy typing. Typically lancet-shaped, the diplococci, with age, rapidly becomes gram negative and spontaneously lyses.

The capsular polysaccharide is immunologically distinct for more than 85 types, providing antigenicity and stimulating B-cell proliferation. The somatic portion contains an M protein characteristic for each type and a C carbohydrate common to all Pneumococci.

Types 1 to 8 and 18 are responsible in adults for around 80% of cases of pneumococcal pneumonia, and for more than 50% of all fatal pneumococcal bacteremia. The virulence of the organism is a function of the capsule which prevents or delays encapsulation in phagocytosis. The normal respiratory mucosa must possess great natural resistance to the Pneumococcus, as 40-70% of humans are carriers of the bacteria. Pneumococcal pneumonias account for about 80% of all bacterial pneumonias in the general human population (6,97).

The problem of capsulation of bacterium and the need for complement fixation has been studied by 2 groups. And, it was found that the encapsulated E Coli required anti-concavalin A or anticon A antibody for the peritoneal mouse macrophage to phagocytize. Con A alone was adequate for

the non-encapsulated variety of E Coli. Polymorphonuclear cells likewise required the anticon A antibody to phagocytize as well as the presence of complement factor.

The author points out that the K-antigen, or K-1 notably, is the capsular serotype which endows a degree of virulence on the bacteria by providing the capsule. This capsule in the absence of antibody locks complement fixation to the bacterial surface probably by masking surface components, such as lipopolysaccharides which normally are capable of activating complement (81,82).

The relative need for opsonins and complement fixation of various microbes is probably the main causative factor for an influx of cells, particularly B-cell lymphocytes. It is the capsule of both E Coli and Pneumococcus that ultimately dictates this response.

Immunoglobulin synthesis within the lung, that is, the local plasma cell production of IgA predominantly, along with IgG, was studied in the rabbit respiratory tract (72). The average IgA : IgG ratio was 2.5 in both normal animals as well as animals infected with Listeria Monocytogenes and Pneumococcus. Interestingly, the animals infected with Pneumococcus had sera containing IgG class antibody, but no IgA antibody; whereas only IgA was found in the secretions from the lower respiratory tract. This is in distinction to the animals which were infected by Listeria who had IgG class within the sera, but had both IgA and IgG

in the secretions. Perhaps the difference in local production was as a result of the persistance of the polysaccharide from Pneumococcus remaining after the clinical infection, although this was not shown in follow-up. For Listeria, the specific antibody production was registered by the 11th day, and for Pneumococcus, by the 8th day.

The importance of complement varies with respect to the microbe under consideration. The complement, of course, enhances macrophage phagocytosis of the bacterial pathogen. A study by Murphy (124), examined the interaction between antibody and complement in phagocytosis by rabbit alveolar macrophages of Staphyloccal Aureus and Pseudomonas Aeruginosa. They found that if the antibody concentration was low in the animal, then the complement concentration became vitally important. Normal rabbit serum was a satisfactory opsonin for Staph. Aureus, but was not one for Pseudomonas. Also, the former was opsonized by both the classical and the alternative pathways.

When concentrations of Pseudomonas immune serum were lowered, either the classic or the alternative pathways would significantly enhance phagocytosis of Pseudomonas. They showed that the alternative complement pathway was less efficient than the classic pathway, but for phagocytizing encapsulated bacterium with polysaccharide, this pathway appears effective in vivo. This complement

pathway may be activated directly by plant or bacterial cell wall polysaccharides or endotoxins, as well as aggregated immunoglobulins. One drawback of the technique utilized in the study was the assumption that the uptake of radioactive labeled bacteria which they used was representative of phagocytosis and not immune adherence as well.

PULMONARY LYMPHATICS

ANATOMY:

Historically, the first description of pulmonary lymphatics was made by Rudbeck in 1653 and later in 1675 was mentioned in the Pharmaceutica Rationalis of Willis (149). Since that time, there have been many contributions to the understanding, but significantly, Wywodzew in 1865 observed that in dogs and horses, lymphatic capillaries were present in the wall of the saccule alveolares.

A hundred years later, in 1947, Miller divided the lymphatic system of the lung into 4 groups, the peribronchial, the periarterial, the perivenous, and the pleural vessels. He emphasized that no lymphatic capillaries occur distal to the alveolar ducts, i.e., in the wall of the alveoli themselves. Although there has been some fine tuning of this concept, it still holds, the major point being that they are all interconnected. Direction of flow is also important for the classification. Lymph flow in the bronchial lymphatics and the periarterial lymphatics is towards the interior of the lungs, while direction of the flow in the perivenous lymphatics is toward the hilus, as indicated anatomically by the position of the valves (133,149).

In 1979, an anatomical study of pulmonary lymphatics was published by a group from Japan, in which they confirmed the classification into 2 systems and 4 groups, that is, the 2 systems were interstitial and parenchymal. The

often cited lack of lymphatics in the interalveolar septum was demonstrated by both electronmicroscopy and light histology. The absence of lymphatics at the septal level is functionally replaced by the extravascular fluid pathways transporting fluid and proteins to the adjacent "juxtaalveolar lymphatics"; these provide the most peripheral part of the respiratory tract with a pathway system (133).

TRANSVASCULAR FLUID FLUX:

In the last 5 years, there has been a great deal of work in attempting to identify the location for the transvascular movement of fluids in the production of pulmonary edema. Two standard types of pulmonary edema are high pressure edema secondary to acute left ventricular failure, and high permeability edema associated, for example, with septic conditions. As we achieve better techniques at examining this transvascular flux, we have a greater understanding.

Studies on the dog lung by continuous weight recording of the intact lung, established a pulmonary capillary filtration coefficient. From increases in the capillary hydrostatic pressures, a critical value (PC) was reached beyond which the lymph flow constant (J-l) was overtaxed, and the lobes continued to gain weight at a more rapid rate. The mathematically derived PC critical is equal to J-l max over KT. This ratio represents a pressure differential across the capillary membrane capable of causing maximum filtration.

To this, was added PT max, which is the tissue pressure maximally registered plus delta, that is, the oncotic pressure differential measured across the capillary membrane. Thus, mathematically, a direct relationship was established between the PC critical and the plasma oncotic pressure (63).

-Site:

The reflection coefficient of urea, glucose, and sucrose was used in an isolated dog preparation to calculate the pore radii in the alveolar capillary membrane. In the alveolus, the pore radius is calculated to be 6-10 Angstroms, whereas the pore radius of the pulmonary capillary membrane is 40-58 Angstroms (see Figure XXXI) Therefore, the pulmonary capillary membrane represents the more highly permeable structure, and the more likely location for possible large fluid transudation (170).

The two pore concept for exchange sites is now fairly well accepted in the transvascular fluid shift. By examining albumin and sucrose flux across the blood-gas barrier, two pores have been postulated - one a small pore pathway and the second involved in direct bulk flow proportional to the concentration difference (179). Further functional definition is needed before one can absolutely match in vitro mathematically derived solute exchange studies to anatomic sites.

Changes are also occurring in the two compartment theory which was adhered to for a long time, but with the advent of radiolabeled solutes, this concept was found inadequate. Now, besides intravascular and intra-alveolar, there is a recognized interstitial fluid space. Staub has to be considered a main force in this area of pulmonary lymphatics in which he has been working for years. Early on, his lymph fistula models, using first dogs, then sheep, allowed good correlation between scintillation scans and measured flow.

Measuring RISA uptake from the lung lobe of the dog, he found the permeability coefficient was more accurate when a third compartment, the interstitium, was added to the alveolar and plasma fluid compartment model (163,164).

The interstitium appears to fill by bulk flow through low resistant channels from the alveolus along hydrostatic pressure gradients. In a possible sequence of edema formation, interstitial edema precedes alveolar flooding. As the interstitium fills with fluid and protein, the interstitial hydrostatic pressure increases until it equals or excedes the pressure in the airway. At that point, fluid and protein leak retrograde back into the small airways and alveoli, at that point reflecting the PC critical as mentioned in the prior study. We will discuss these concepts more in the section on pulmonary edema.

For years, researchers have pursued the concept of transitional vessels as the site of transvascular fluid movement via the "pore system". Macklin and Permutt over 20 years ago tendered the idea of intra-alveolar and alveolar vessels. When examining blood volume, they considered the pulmonary vessels to act as if composed of 2 compartments. One that expands with a rise in lung volume and the other that is gradually compressed during the same maneuver (129,179).

This line of investigation led to the more recent discoveries by Nicolaysen that the site of exchange does indeed appear to be in transitional vessels that behave as both alveolar and extra-alveolar. He demonstrated this by examining isolated plasma-perfused rabbit lungs which were kept completely in zone 1 condition; that is, the alveolar pressure was greater than the vascular pressure at all levels in the lung (129). A decrease in pleural pressure at constant alveolar and vascular pressures resulted in an increased rate of filtration and a decreased rate of re-absorption. On the other hand, an increase in the alveolar pressure at constant pleural and vascular pressures led to a decreased rate of filtration or an increased rate of absorption. The localization of the transitional vessels is consistent with the so called "corner vessels" at the alveolar septal junctions. Here, the vessels are known to remain open in the zone 1 lungs as shown by rapid-freezing technique. Anatomically, these vessels are distinct from

alveolar septal capillaries which close at equivalent alveolar pressures.

The examination of edema formation in the lung has been advanced by radio-isotopic investigation that has utilized the 3 compartment concept. For example, Staub not only correlated lymph and protein analysis with scintillation transvascular protein flux, but he selectively labeled the involved spaces. Albumin I 131 which is diffusable, is followed for wash in or out presumably capable of transiting the pores, and 51-Chromium tagged erythrocytes non-diffusable and therefore will act as a relatively stable background label (64,165).

A group from Oxford cannulated the right lymphatic duct in a dog and correlated rapid exchange of the radiolabeled albumin between the plasma and the interstitial space (139). This cannulation in the dog's right lymphatic duct had a mean flow of 2.8 ml per hour with a baseline lymph : plasma protein ratio of 3.4 to 5.9, noting that the measurements were taken at the same transpulmonary pressure to avoid any variation. Two points that the group makes are that an arbitrarily postulated 2 compartment model was unsatisfactory for calculations of transcapillary albumin flux if the size of the interstitial space varied. In fact, this size does seem to vary and therefore, the 3 compartment model is more physiologically valid. Secondly, they pointed out that the presence of a chest wall in man and close-chested animals

precludes the direct application of this method because of interference. However, the group hastened to add that isotopes with emission of gamma rays of 2 different energies, such as I 123 may be used to study the deep organs in the future.

-Alveolar Absorption:

The effect of absorption of material from alveoli into the system is increasingly recognized as an important physiologic action with significant clinical implications. Certainly the study that we have made involving the introduction of aerosolized microbes has to have some understanding of this process. The possible exchange mechanism was discovered in one study as the pulmonary blood. The absorption of albumin from the alveoli of anaesthetized dogs was evaluated by radio-isotope, after cannulating the thoracic and/or the right lymphatic ducts (117).

Albumin absorption was 7 to 11 times greater by the blood than by the lymphatics, and the rate limiting step in the removal of protein was from the alveoli interstitial space rather than passage through the alveoli epithelium. Although the latter did demonstrate lymphatic removal of the alveoli albumin, it represented only minor amounts in terms of the total absorption. The calculated derived ratio of regional blood uptake to the lymphatic uptake was 10.7 to 1 or with a relative efficiency expressed as 37 to 1.

Absorption of the intact protein molecule albumin radiolabeled with I 131 has been shown in the intact dog lung from alveolus to the vascular compartment. This demonstration of intact antigenic protein particles absorption has some physiologic importance as it represents a conservation of solutes thay may escape into the air spaces. This may also represent an important mechanism for allergic reactions in the lung as well as the formulation of antibodies (10).

Finally, the transalveolar uptake and utilization of aerosolized glucose is interesting from a metabolic as well as absorption point of view. Comparisons were made of the production of Carbon-14 labeled CO2 incorporation into the lung lipid material. The maintenance of high energy phosphate levels in lung tissue confirmed that the substrate could be utilized from the airway side of the isolated rabbit lung (78).

PULMONARY EDEMA:

-Introduction:

Pulmonary edema may represent the final common pathway in respiratory failure from many different etiologies not the least of which from our point of view is the introduction of bacterium. In fact, the patient who is proven to be septic with positive blood cultures and who does not have some pulmonary complication, such as edema or even shock is uncommon. Besides the specific organism, the handling

of resuscitation, the underlying cardiac or pulmonary disease, as well as overall nutritional status become relevant in managing this patient. Cognizant of the fact that our work primarily centers on the effects of bacterial infusion into relatively normal lungs, the interaction between many factors in the formation of pulmonary edema led us to review the subject, both in the experimental and in the clinical literature.

There are two basic types of pulmonary edema: the pulmonary capillary hydrostatic pressure edema or so called "high pressure", and altered permeability edema or "low pressure". As well, there is a small sub-group classified as "lymphatic failure". Of the high pressure type edema, the most common is the cardiac or left ventricular failure model. Non-cardiac causes are the occlusive diseases with lesions located within the pulmonary veins and venules, and sometimes seen on post-mortem. Fluid overload in resuscitation also represents a type of high pressure edema. A third group is that of lymphatic insufficiency and major causes that are known, silicosis and carcinomatosis, both result in chronic obstruction of lymphatics predisposing to pulmonary edema. Our main interest for the purposes of our studies, however, is the second major type of pulmonary edema from altered permeability to the transcapillary fluid flux that we discussed earlier.

-Lymphatic Cannulation:

Much of the knowledge about pulmonary edema has been gathered, as we have seen, from the non-invasive techniques of radio-isotope labeling and solute transcapillary flux. Functional studies, utilizing lymphatic cannulation, however, were needed to make in vivo correlation with the various states of pulmonary edema.

The development of lymphatic cannulation began with the early work of Drinker (1940's), who emphasized the right lymphatic duct in making valid studies of "most of" the lung fluids (149). Since then, Staub and his group from San Francisco, have developed a sophisticated chronic lymph fistula technique. From this and other work, the right lymphatic system does seem preferable to examine for a number of reasons.

After the bronchopulmonary nodes, Staub's group demonstrated that all efferent lymph vessels of the lung, except for the lymphatics of the left apical area, drain into the right lymphatic duct (54,177,178). The left apical area drains into the thoracic duct. In fact, because this was largely ignored with studies on the thoracic duct in the past, particularly in Drinker's earlier work, his data has to be considered inconclusive. Lymph in the right lymphatic duct is mixed with pulmonary and pleural as well as pericardial, and right upper extremity lymph, as shown by radio-isotope methods.

Vreim and Okuda developed a method of cannulating the right lymphatic duct using the cephalic vein as a landmark, a modification of which we used in our pig studies (177). In another paper by them, they showed that in dogs, lung lymph to the thoracic duct and to the right lymphatic was divided 50% to one and 50% to the other (133). They demonstrated this using simultaneous cannulation, ligating the thoracic duct above the diaphragm and inducing alloxan pulmonary edema. However, the right lymphatic duct was shown to be more accurate in that 8 of 8 dogs had increases in the right lymphatic duct flow with the pulmonary edema, while only 4 of 8 had increases in the thoracic duct. Again, they cannulated the right lymphatic via the cervical lymphatic from the neck. Gee's study confirms that the right lymphatic duct did indeed increase in lymphatic flow by greater increments averaging sixty-fold increases, while the thoracic duct had only thirty-fold increases when a fluid challenge equivalent to 30% of a dog's body weight was given (54). They used the technique of Vreim and Okuda, cannulating the right lymphatic duct.

The present so called "Staub preparation" is the chronic lymph fistula in the sheep which is placed in the right caudate mediastinal lymph node efferent, and involves a thoracotomy. A combination of this model with radioisotope studies provides us with functional information on fluid and solute flow in the compartments of the lung.

One such study serves to illustrate this and provides us with some basic figures.

A Staub sheep model preparation was used to measure pulmonary vascular pressure, lymphatic flow, lymphatic to plasma protein ratio, using radio-labeled albumin as a washout technique. In one preparation, Pseudomonas Aeruginosa bacterium was infused intravenously to create the increased permeability type pulmonary edema; and in another, a left atrial balloon was placed and inflated to cause high pressure edema. The mean results are the following: measurements of the micro-vascular pressure in cm of water, lymph flow in ml per hour, lymph to plasma protein ratio and tracer equilibration expressed as half-life in hours was 10.7, 5.3, .87 and 2.9 respectively for controls, high pressure edema was 25.4, 16.1, 0.63 and 2.2 respectively; and finally, increased permeability was 12.9, 37.3, 0.77 and 0.7 respectively. Understanding the pathophysiology, the high lymph to plasma protein ratio for the controls is a reflection of the important role the lymphatics perform in retrieving and returning proteins to the general circulation (45,114,176). We will examine the specific effects of bacteria on the formation of pulmonary edema in a later section (see Sepsis).

-Lymphatic Reserve:

The concept of high or low pressure edema may have less clinical relevance than the concept of "lymphatic pump failure". The transcapillary fluid and protein balance is one of constant exchange in both normal and abnormal

situations with the rate limiting step lymphatic removal of fluids and protein solutes. This solute retrieval and return to the systemic circulation may have the largest consequences for the formation of clinically evident pulmonary edema. The concept of the lymphatic pump in combination with the interstitial matrix preventing edema formation in normal as well as abnormal situations implies a reserve capability when the system is taxed - a safety factor as it were.

The interstitial pulmonary space, in effect, represents an important reservoir located so that it allows the accumulation of fluid and protein at a site that is remote from the primary gas exchanging sites in the lung (21,65). The interstitium itself is composed of collagen, mucopolysaccharides and other structural macromolecules which are linked by covalent, electrostatic, and hydrogen bonds as well as by mechanical ties. This 3-dimensional matrix provides a sponge-like space between parenchymal cells for the containment of the solvent and the solute molecules. Any increase in interstitial pressure leads to an increase in lymphatic flow rate allowing more rapid removal of both water and protein from the interstitium.

A major factor in the compensatory power of the vessels to prevent edema, is the sensitivity of the lymphatic pump to changes in interstitial pressures or volume. Myogenic response or control of pumping is dependant on the beat

frequency and stroke volume both of which are variably graded in proportion to the degree of stretch of the lymph vessel itself. Pressure dissipation by the lymph pump minimizes fluid accumulation in the interstitium when capillary pressures rise.

Brigham in a normal sheep preparation showed that with interstitial pressure rises of as much as 10 mm mercury, there is no change in measured lymphatic flow. In this case, he calculated that the sum of interstitial matrix and oncotic pressures changed by 10 mm mercury to keep a balanced steady state. If, however, the lymph flow increased in response to small increments of the interstitial fluid, the sum of the two interstitial forces may change by only 5 mm mercury. (21)

The second important facet of lymphatic compensation is the protein "washout" from the interstitial matrix by an increased efflux and dilutional effect, reducing interstitial oncotic pressure. When fluid accumulates in the interstitium, the oncotic pressure falls by simple dilution because a greater portion of the interstitial water is now available for proteins to be more diffusely distributed.(65)

According to the individual situation, the matrix may in fact vary in being soft or firm, absorptive or non-absorptive. The interstitium tends to be generally compliant in areas, such as the lung, where an increase in interstitial pressure

would cause a severe dysfunction. Steric hinderance may also cause the matrix to inhibit or discourage the entrance of plasma proteins.

In summary, the margins of safety are provided by 1) myogenic compensation which is sensitive to the increased pulmonary capillary resistance as well as the decreased capillary surface area, 2) to the interstitial compensation secondary to increased interstitial fluid pressure and dilution of the interstitial pressure, and 3) the lymphatic compensation with an increase in fluid removal and a washout of the interstitial protein (21,147).

The concept that pulmonary lymphatics have inherent safety factors is a comforting one. In reality, however, there are numerous clinical situations or conditions that appear to overwhelm this faculty of lymphatic fluid and protein removal. For practical purposes, the lymphatic reserve cannot be externally manipulated other than to keep a highly compliant lung, to increase the reservoir. Treatment, at this time, must continue to focus on the underlying disease process.

-Toxic Inhalants:

Toxic inhalants are known etiologic factors for the high permeability type of pulmonary edema. These include, for examples, phosgene gases, nitrogen oxide which is found with silo-filler's disease, and paraquat which is used in

marijuana spraying in Mexico and is so injurious to marijuana smokers. The inhalants have a direct effect on the alveolar and endothelial cells (147).

Two inhalants which we have discussed with regard to macrophage and cilia effects are cigarette smoking and oxygen. The alveolar capillary membrane essentially consists of the epithelial layer, the endothelial layer, and the interstitial space, as we have seen. Up to 90% of the resistance to the transcapillary flux and diffusion of hydrophilic molecules is in the epithelial or the alveolar portion, which is consistent with the work of Taylor and others (170,179). Early changes in alveolar permeability can be demonstrated with the use of technetium-labeled albumin in cigarette smokers (99). Less than a pack-a-day smokers had an increased alveolar diffusion rate versus that of the non-smoker; this measurable abnormality may be a predisposing factor for pulmonary edema development.

Pulmonary oxygen toxicity appears to be as a result of high concentrations causing peroxidation of unsaturated fatty acids in cell membranes and further cytotoxic effects through free radical damage. A study performed on Staub-prepared lambs breathing 100% oxygen for 5 days demonstrated that lymph flow doubled, protein flow increased by 131%, and as well, radioactive tracers showed an increase in pulmonary microvascular permeability (20). There was also an increase in pulmonary vascular resistance over the 5 day period of

oxygen therapy, a finding that appears to be an almost uniformly consistent one with pulmonary dysfunction of many differing etiologies.

The possible hyperoxic injury to the Type-2 alveolar pneumocyte could also predispose to pulmonary edema by the diminishment in surfactant. Surfactant is probably better known for its surface fusion effects than as a previously discussed bactericidal substrate (see Phagocytosis). The action of alveolar surface tension effect is to create a vector of force into the alveolus wherever the alveolar surface is conven. For this reason, intra-alveolar edema tends to develop at the corners. Likewise, it is for this reason that the surfactant action more equitably distributing the tension over the entire surface, helps to prevent collapse and formation of edema.

-Illness and Complication:

Illnesses and complications secondary to their treatment can cause high and low pressure edema and they include the following.

Pulmonary embolism causes a release of vasoactive substances resulting in an increase in capillary permeability. Serotonin is released in the largest quantities, thus producing pulmonary venous constriction with large secondary fluid transudation. Cardiopulmonary bypass has multiple factors including neurogenic as well as micro-emboli with the release of toxic

substances. Metabolic deprivation during long cardio-pulmonary bypass must also be considered as a possible etiology (119). A type of DIC or diffuse intravascular coagulation may play a role in these and other situations, as it has been shown to cause diffuse endothelial damage. As well as extracorporeal circulation, DIC can be secondary to immune complex diseases, eclampsia, amniotic or prostatic fluid embolus, and from sepsis and endotoxemia.

Aspiration pneumonitis, in the obtunded incapacitated patient, causes permeability pulmonary edema. Animal studies show that after instillation of acidic solutions with pH varying from 1.5 to 4.5, a pH of 1.5 to 2.5 showed the most significant edema changes compared to those of the other groups (58,59). Pretreatment with steroids in the quantity of 30 mg of prednisolone per kilogram body weight revealed no benefit with a pH less than or equal to 2.5 in preventing alveolar epithelial permeability. This was studied using the transcapillary solutes albumin which measures 35 angstroms and dextran which measures 100 angstroms in diameter. In animals either pretreated 30 minutes prior or 30 minutes after, there was no improvement or decrease in resultant permeability.

Lastly, acute left heart failure in compromised cardiac patients by over-hydration is the most common cause of high pressure pulmonary edema. Thankfully, this type is probably the most amenable to treatment.

-High Velocity Blood Flow:

Staub recently described high linear velocity in pulmonary blood flow as an additional etiology of permeability edema (164). The effect is secondary to injury of the micro-vascular endothelium. He cites recent reports of humans who develop acute pulmonary edema after exertion in high altitude in whom the right pulmonary artery was found to be congenitally absent. The ramification of this concept is not just high altitude or neurogenic edema, but also to the more common problem of multiple pulmonary or micro-emboli. Localized edema may develop in areas of perfused lung that would receive relatively more blood flow from a shunting effect.

In an experiment to follow this up, he and his colleagues resected all but the lower lobe of sheep and obtained an increase in interstitial edema with a protein content consistent with high pressure edema in the remaining interstitial spaces. This has fairly obvious clinical significance for the post-operative management of the pneumonectomy patient. The remaining lung tissue must therefore rapidly accommodate a greatly increased amount of blood.

-Miscellaneous:

Other causes of high permeability edema are mentioned briefly and include the following (21,56,147).

High altitude type edema, the Andeans call it "Siroche",

to date has no good explanation although a neurogenic etiology is possible, secondary to the imbalance of alpha and beta-adrenergic activity produced by an increase in CSF pressure. Also, hypervolemia secondary to relative hypoxia with transient intravascular coagulation is suggested. Heroin overdose is again possibly a neurogenic etiology as post-mortem cerebral edema is found in a large percentage of addicts with both enteral and parenteral administration.

The release of vasoactive substances, such as histamine, serotonin, kinines, prostaglandin all result in an increased permeability of the bronchial venules. These are, of course, mostly involved with the anaphylactic situation and endogenously controlled in the lung as we discussed in the section on hormone capacitance and prostaglandin mediation. The causes of this release may also be a non-reagin reaction like hypersensitivity pneumonitis, seen in medical conditions, such as Systemic Lupus erythematosis, Good Pasture's Disease, and also with sensitization to leukocytes secondary to multiple transfusions. This last situation of shock lung evolving from massive injury and hemorrhagic shock with massive resuscitation including transfusion is not uncommon in surgical care and management. This topic will be covered in the section on Hemorrhagic Shock.

-Prostaglandin Mediation-

The interaction of the opposite effects of prostaglandins

may be of paramount importance in the development of fluid and protein imbalances within the lung, as we have seen in an earlier section. For example, the depressed inactivation of prostaglandins demonstrated in the rat after being given endotoxin has significant implications for the pulmonary picture in gram negative sepsis. (97)

Of clinical interest is the fact that heretofore the hemodynamic response to positive pressure ventilation has been attributed to a decrease in venous return. By pretreating animals with aspirin, it is possible to partially avert the anticipated decline in systemic blood pressure (32,48,135,113). Aspirin, of course, acetylates cyclo-oxygenase and is a well recognized inhibitor of prostaglandin synthesis activity.

Animals pretreated with aspirin showed a significant reduction in embolus-induced pulmonary vasoconstriction. The embolized guinea pig lungs released high concentration of prostaglandins and rabbit aorta contracting substance which has since been identified as thromboxin. Finally, increased prostaglandin release has been detected in edematous lungs further implicating the substances in the cause and effect of ARDS.

Paretti and his group studied a patient with congenital deficiency of cyclo-oxygenase and found that low dose aspirin was more effective than high dose at inhibiting

the production of thromboxin A, a potent inducer of platelet aggregation. At higher doses while it did inactivate thromboxin A2, it likewise reduced the vascular concentration of the anti-aggregatory prostacyclin PGI-2, thus producing a thrombotic tendency (135).

Prostaglandin balance was also implicated in another animal study in which the clinical picture of ARDS was created by infusion into sheep of activated serum incubated with zymosan (98). The production of a relative leukopenia and pulmonary leukostasis, with resultant increase in pulmonary vascular resistance and decrease in pulmonary arterial oxygen are typical findings with ARDS. When the animals were pretreated with sulfinpyrazone, a known prostaglandin blocker, the pulmonary effects were prevented. There was no evidence of a direct pulmonary vasodilator effect, nor was complement activation effected, and lastly, there was no change in platelet number or response to ADP to account for the beneficial effects of sulfinpyrazone. Therefore, the benefits were felt to be secondary to the drug's effect as a prostaglandin blocker.

-Diagnostic Techniques:

The clinical interest in the formation of pulmonary edema has led to the development of quantification techniques that might in the future be applied to the human situation. The first is the measurement of lung density utilizing a beam of gamma rays to quantitate lung water by comparison to previously established lung density readings for normal

and abnormal situations. The technique utilizes the transthoracic gamma ray cobalt-57 and has been confirmed by dog experiments to accurately reflect the water changes within the lung during acute pulmonary edema (53,156). This technique certainly has promise for the clinical application as it is non-invasive and appears to be a relatively accurate technique if good controls are first established.

Another method slightly more sophisticated and one which is not clinically applicable at the current time involves enzymatic tracers (158). The tracers which are peroxidatic are I.V. infusions of varying size molecules which function as probe molecules for vascular permeability. The author, Simionescu, discusses the complexities taking into account molecular weight, peroxidase activity, molecular charge, toxicities involved, cross-linking with tissue and the optimal pH and temperature. Three major problems are identified with the technique before it can be used in clinical situations, and they are: 1) the need for quantitation, 2) the need for tracers with dimensions less than 20 Angstroms and 3) the need for tracers which are more physiologic and frequent inhabitants of plasma and interstitial fluid. So that to date, this technique can only be considered experimental, and although appealing from a molecular point of view, is largely superceded by radio-labeled solutes.

A group from the Intensive Care Unit at the San Francisco

General Hospital recently developed a bedside technique to measure extravascular lung water in following patients who suffered from pulmonary edema, particularly those with adult respiratory distress syndrome (107). The technique involves the utilization of thermal-green dye double indicator dilution technique, and requires a 5-French femoral artery catheter with attached thermistor, and either a CVP or PA catheter. The lung water is calculated as a product of the thermocardiac output and the difference in the mean transit times between thermal and the dye curves. The results correlated well with any autopsy findings of lung water as well as such clinical parameters as the chest X-ray, the PaO2, the PA wedge, and the pulmonary compliance.

The examination and classification of pulmonary edema in clinical situations, as to high or low pressure types, has confirmed the experimental concepts of transvascular flux. Patients with left ventricular failure or in whom volume overload was a primary cause of edema were compared to patients who exhibited the high permeability edema (24). Patients in this latter group had suffered from shock, aspiration, bacteremia, near drowning, coagulopathies, drug overdoses and pulmonary emboli as well as smoke inhalation. It was found that the edema fluid to plasma colloid osmotic pressure ratio for the multiple cause (or high permeability group), was significantly higher than the left ventricular failure group with a mean of

0.91 versus 0.51. As well, the group with the high permeability edema had normal pulmonary wedge pressure measurements. As long as the plasma colloid osmotic pressure was maintained with colloid replacement, the rapid transvascular fluid flux continued primarily due to the increased filtration at the alveolar capillary membrane due to its permeability. On the other hand, when pulmonary edema develops from high pressure, the membrane remains an effective barrier to proteins, particularly the larger ones such as globulins. This was confirmed by a relatively lower and more stable colloid lymph to plasma ratio (56).

The other side of the spectrum, the high pressure type edema, was examined in several clinical populations and the findings confirmed the transudation theory initially elaborated by Guyton. That is, the fluid began to transude into the lungs when the left atrial pressure was increased to greater than 25 mm mercury if the plasma protein concentrations were normal. If the protein concentrations were reduced to half through plasmapheresis or hemorrhagic shock with a replacement of non-colloidal substances, the transudation began at 11 mm mercury (123,166). This showed that the severity of pulmonary edema was proportional to the gradient between the plasma colloid osmotic pressure and the hydrostatic pressure in the pulmonary edema formation.

In another recent report, 26 patients with acute myocardial infarction, 14 of whom developed pulmonary edema, were studied (33). To predict the high risk of pulmonary edema, the gradient of the pulmonary wedge pressure to colloid osmotic pressure was less than or equal to 9 mm Hg. This ratio was utilized in the clinical management and therapy in patients after the onset of high pressure edema, in this case secondary to acute left ventricular failure. The concept is essentially a clinical extrapolation of Starling's forces that Guyton and others elaborated.

-Sepsis:

As we have seen from the clinical study by Fulton and Jones (50), sepsis is the single most common etiologic factor in post-traumatic mortality. As part of the multiple organ failure pulmonary sepsis, considered for years "the old man's friend", has a very high lethality. The mechanical diffusion barrier in the alveoli with edema fluid is the most obvious lethal mechanism. Depending on the type of bacteria, systemic circulatory collapse may occur as well as the lung effects. Death, if it occurs, has a multi-factorial etiology.

Organisms vary as to their lesser or greater pulmonary edema potential, with gram negative bacteria a standard to which comparisons are made. The hemodynamic and pulmonary effects of infusing live E Coli intravenously as compared to purified endotoxin into dog and monkey demonstrated that live E Coli was more effective in eliciting a damaging effect on the

lung (154). This was reflected by an increased pulmonary capillary pressure of 10 mm mercury with the live bacteria versus 7.4 mm mercury for the endotoxin studies, and as well, the former exhibited a greater decrease in surfactant production. Histologically widespread alveolar and endothelial cellular damage with florid edema and hemorrhage into the alveoli as well as endothelial disruption, was greater with the E Coli.

Moss and his colleagues from Chicago also examined the effect of gram negative bacteremia on the etiology of pulmonary edema (145). Studying the live baboon, they examined the interaction between sepsis alone, infusing live E Coli intravenously at 2.7 to 5.4 x 10^9 organisms per hour, plasmapheresis alone, and plasmapheresis and sepsis together as the possible etiologies of pulmonary edema. The effect of plasmapheresing the animals was to decrease their effective colloid osmotic pressure significantly.

The colloid osmotic pressure decreased by 47% with plasmapheresis along, 60% by plasmapheresis and sepsis, and 32% by sepsis alone. The wet-to-dry lung weight ratios on all 3 groups were similar with no significant differences shown and during the experiment, the pulmonary artery wedge pressure gradient remained the same in all groups. The conclusion in this study was that E Coli sepsis alone does not account for the formation of pulmonary edema,

but is part of a cumulative effect with other factors, such as malnutrition, previous lung, or cardiac injury.

In some slightly more sophisticated work, Brigham and Weim compared high and low pressure (high permeability) edema using a Staub sheep preparation. The high pressure variety was created by mechanically causing left atrial outflow obstruction using a left atrial balloon, and the high permeability type by intravenous infusions of histamine, Pseudomonas bacteria, and E Coli endotoxin itself. (22)

These microbes were infused at a rate of 0.2 to 1.33 micrograms per kilogram over a 30 minute period, and the animals followed for 6 to 8 hours. Three transport coefficients were required to characterize the properties of the pores involving filtration, reflection and permeability coefficients.

Assumptions that were developed by Staub's early work and used in this study are that a significant fraction of the protein fluid and tracer molecules move through the same pore structure. That the capillary membrane undergoes passive transport due to a hydrostatic concentration gradient. That the interstitium has concentrations of solutes identical to concentrations in the lymph draining the interstitium and that this is drained by non-sieving lymph ducts (163).

In contrast to Moss's work, he found that E Coli endotoxin caused the greatest increase in lymph flow rate (30 to 45 ml per hour), as well as the highest protein content. Analysis of 8 protein fractions revealed that the endotoxin caused a much greater relative clearance of the larger fractions than did the other substances. In contrast to the increased pressure pulmonary edema, the edema induced by histamine, Pseudomonas, and endotoxin all resulted in an increased permeability to the proteins of molecular radius 35.5 to 96 angstroms, and the greatest being with endotoxin (22). These findings are all consistent with a 3-compartment model.

Fifteen patients with cardiac (high pressure) and 15 patients with septic edema (high permeability) were examined using radio-isotope technique (76). The transvascular washout in the lung was shown using 2 radio-labeled solutes - albumin with a molecular weight of 69,000 and DTPA with a molecular weight of 500. Patients were studied over a 4 hour period by scintillation counter; and additionally, pulmonary capillary wedge pressure and colloid osmotic pressure gradient were obtained. The findings are that septic type edema causes a greater movement of both small and large molecular weight solutes from the blood into the pulmonary edema fluid than does the cardiac edema. The information is significant as good clinical correlation with already accepted concepts.

The depressant effect of pulmonary edema, any type, on lung antibacterial capability was first noted in 1934 in a study we have cited from Bellevue Hospital. In a series of 86 cases of secondary Pneumococcal pneumonia, the most common antecedent disease, excluding alcoholism and respiratory infection, was found to be congestive heart failure. Laforce in the early 1970's examined mouse and rat lungs for this effect after inducing pulmonary edema of both types; using ANTU (alpha-napthyl-thiourea) in mice and surgical constriction of abdominal aorta in rats (104).

Four hours after aerosolization with radio-phosphorus tagged Staph. Aureus, the lungs were homogenized, and cultured. In both models resulting in the pulmonary edema situation, there was a decrease in the anti-bacterial activity as measured by retained viable organisms as well as measured radioactive level. Significantly, the dysfunction was relative to the amount of intrapulmonary fluid accumulated and quantified. Hypoxia does not appear a factor as exposure to 60% oxygen did not reverse this impairment. Possible theories are an interference with surface interaction between the phagocyte and the microbe, and the alteration of pulmonary surfactant with the pulmonary edema. In the ensuing 8 years, development and addition of an in vitro bactericidal test of the pulmonary macrophage, performed after the ANTU production of edema showed a definite functional defect.

-Hemorrhagic Shock:

The formation of high permeability edema with the so called shock lung has etiologies with the underlying perfusion insult, as well as the type and method of resuscitation that ensues. The syndrome that often becomes evident in patients who receive greater than 8-10 units of banked blood might also be called transfusion lung, as we have earlier mentioned.

The factors that have been incriminated with transfusion reactions causing a picture of shock lung are multiple, but there are currently two dominant factors. The first is related to the infusion of microemboli within the stored banked blood; and the second to platelet aggregation within the lung beds, as well as a leukocyte sensitization causing a complement activated reaction within the lung beds after multiple transfusions. This information is from work done in rat lungs after bleeding 25% total body weight and then replacing shed blood in equivalent amounts (11,147).

The concept of hemorrhage induced lymphatic failure is examined by another group as the most important etiology for pulmonary edema. Using the lymph fistula technique in sheep, Malik demonstrated that the net transvascular protein flux actually decreased during the period of hemorrhagic shock, but that pulmonary edema developed (110). Therefore, he concludes that the edema was not due to an increase in the lung vascular endothelial

permeability, but more likely due to hemorrhage-induced lymphatic "failure".

The endothelial cell represents approximately 30% of the total extravascular tissue mass within the lung and may represent an important site for accumulation rather than the interstitial space. Unlike the interstitial space, the endothelial space is not drained by lymphatic effluents. Finally, swelling, which is noted in the endothelial cells, may in fact be the basis of a rise in the pulmonary vascular resistance pressure which we have noted is associated with many situations of respiratory failure.

-Peep:

Any discussion of the concept of pulmonary edema particularly in the clinical situation has to include a brief discussion of the effects, if any, of positive end expiratory pressure ventilation or PEEP. Three separate studies examined the effectiveness of up to 10 cm of water on non-cardiac pulmonary edema in animal models. Hopewell found that the measurement of pulmonary extravascular water as measured by wet-to-dry lung weights in dogs was not significantly different with or without the 10 cm of PEEP. He initiated pulmonary edema using alloxan (86).

Staub effectively found the same result with E Coli induced edema; that positive pressure did not change the trans-vascular lung fluid balance. The unchanged filtration rate

with and without PEEP is explained by the redistribution of pressures. The rise in pulmonary vascular pressure from PEEP is blanced by an equal rise in the peri-microvascular interstitial fluid pressure (163).

In a final paper, Bredenberg and Webb examined dogs in whom they studied the high pressure type of pulmonary edema created by left atrial balloon. PEEP in this condition did not effect lung lymph flow, but it did increase effective lung volume, as well as pleural pressure and pulmonary vadcular pressure. Any effect in raising the PaO2 that it may have is probably related to the effective increase in the gas exchanging alveoli (19).

-Colloid versus Crystalloid:

The controversy of whether to utilize colloid or crystalloid in the resuscitation of the shock patient, is hotly debated in surgical specialties. In Staub prepared sheep: before, during, and after hemorrhagic shock, the effects of changes in plasma oncotic pressure on the pulmonary transvascular fluid filtration was studied (37). Fifty percent of the blood volume was lost over a 3 hour period, in unanaesthetized sheep. Resuscitation was with either shed blood or Ringer's lactate sufficient to increase the left atrial pressure and the cardiac output to a baseline level.

Lymph flow increased by 115 to 120% in both situations,

utilizing either Ringer's lactate or shed blood. Plasma oncotic pressure decreased by 50% with Ringer's lactate infusion and increased with blood to a gradient less than 4 mm than blood. The compensatory mechanism appears to be a similar decrease in the interstitial oncotic pressure to keep the gradient in a steady state. Therefore, the effective transvascular flow is unchanged. Situations where the compensatory ability is absent because of "lymphatic failure" or "interstitial saturation" is where pulmonary edema or the so called shock lung picture can easily develop secondary to transfusions (37,38).

EXPERIMENTAL

INTRODUCTION:

> In basic research, everything is just the opposite. What you need at the outset is a high degree of uncertainty; otherwise, it isn't likely to be an important problem. You start with an incomplete roster of facts, characterized by their ambiguity; often the problem consists of discovering the connections between unrelated pieces of information (172).

The motivation to study lung immunology is based on its enormous clinical significance, as has been stressed throughout the review sections. Daily a physician in clinical practice is faced with just the problems we have discussed. Not uncommonly, one has the realization that much is unknown about the lung, particularly with respect to local immunology. The field is as large, as it is interesting, and we therefore chose to focus our attention on the single variable of atelectasis and its effects, if any, on bacterial clearance, lymphatics, and all mediated immunity. At the outset, we enumerated the model characteristics, particularly simplicity and reproducibility for which we would look during our review.

-Study Objectives:

The first question we asked was does atelectasis in fact impair bacterial clearance as compared to normal well-aerated lung tissue in an otherwise similar condition? If the answer was positive, we then wished to examine several possible etiologies. The first was any resultant

dysfunction of the alveolar macrophage whose importance in coordinating local lung immunology has been well supported by the literature. The second was the concept of exit pathways available to the lung, allowing rapid and unhindered removal of ingested and partially ingested bacteria. These pathways take the form of lymphatics, blood, and mucociliary.

The capability of testing the AM (CMI) function was developed in our laboratory during the study period. This necessitated a thorough review of the current literature, as well as more than a little trial and error in developing our techniques. Recognizing the importance of the subject, our laboratory is actively continuing research in this area of lung immunology.

METHODS AND MATERIALS:

-Model Preparation:

Twenty-five conditioned Yorkshire swine of both sexes were used, weighing approximately 16-25 kg. Animals were fasted on the night before surgery, and were eliminated from study if they had been on antibiotics for any reason, or showed signs of infection, particularly respiratory. They were anaesthetized by intravenous injection of sodium pentobarbital (30 mg/kg); and additional amounts were administered as needed during the procedure.

An endotracheal tube was placed after an initial broncho-

pulmonary lavage, and the animal ventilated on a Harvard volume respirator with 21% oxygen and a tidal volume of 15 ml/kg. At no time was a general anaesthetic administered. Preparatory surgery required a 2-3 hour period of ventilation.

A number 10 PE tube was introduced into the right pulmonary lymphatic duct using the technique of Vreim and Okuda mentioned earlier. Lymphatic tributaries into the ampulla was ligated as needed by the small Weck clips. Tubing was connected to a tuberculin syringe which intermittently required a 0.1 ml flush of heparinized saline to maintain patency. The wound was closed to accommodate tubing and adaptor for overnight monitoring.

All animals received a total of less than 1,000 ml of Ringer's lactate intravenously over the 24 hour period.

-Baseline Measurements:

Figure I is the worksheet we developed on each animal experiment, and it contains the samples and measurements that we obtained. In the first 8 experiments, we obtained these at baseline or 0 hour, and hourly up to 6 hours. For the final 17 experiments, we obtained 0 hour and 24 hour samples and measurements.

PIG# _____ Kg _____ SEX _____ DATE _____

PROCEDURE _____ XRAY? _____ TIME, BEGAN _____ ENDED _____

MICROBIOLOGY:

ORGANISM _____ QUANTITY _____

METHOD GIVEN _____

CULTURES

OH R _____ L _____

24H RL _____ LL _____

RU _____ LU _____

LYMPH OH _____

24H _____

BLOOD OH _____

24H _____

TRACHEA OH _____

24H _____

HISTOLOGY: LUNG R: OH _____ 24H _____ (24H _____)

L: OH _____ 24H _____ (24H _____)

LYMPH OH _____

24H _____

OBSERVATIONS: _____

MACROPHAGES

OH R: NUMBER _____ MORPHOLOGY _____

CHEMOTAXIS _____ PHAGOCYTOSIS _____

L: NUMBER _____ MORPHOLOGY _____

CHEMOTAXIS _____ PHAGOCYTOSIS _____

24H R: NUMBER _____ MORPHOLOGY _____

CHEMOTAXIS _____ PHAGOCYTOSIS _____

BACTERICIDAL _____

L: NUMBER _____ MORPHOLOGY _____

CHEMOTAXIS _____ PHAGOCYTOSIS _____

BACTERICIDAL _____

OBSERVATIONS: _____

LYMPH - PROCEDURE _____ FLOW RATE OH-6H _____ 24-26 H _____

PROTEIN _____ SERUM PROTEIN _____

Figure I: Worksheet used on each 24 hour experiment.

-Bronchopulmonary Lavage:

Bronchopulmonary lavage was performed using the Jackson straight bronchoscope to lavage and aspirate 25 cc of normal saline into the right lower lobe. Specimen was retrieved in sputum sample trap and immediately transferred to a polypropylene tube and placed on ice. In the inital 8 experiments, samples obtained from both lungs did not vary significantly, and in the final experiments the assumption was made that the one specimen from a normal animal represented a bilateral baseline. The lavage specimens were routinely examined by light histology and occasionally (4 animals) by electron-microscopy. Cell differentials were done on all specimens as part of the preparation for AM function testing which we will discuss in a later section.

-Lymph Flow:

The lymph flow over a 3-4 hour period was measured and flow expressed as ml/h. Specimens were routinely obtained for light histology using a hematoxylin and eosin (H and E) stain and a Geimsa stain to observe cellular content and morphology. A specimen was likewise submitted for culture, and for total protein placed on ice immediately.

One animal was pretreated for 24 hours with 100 mg indomethacin suppository B.I.D. as part of our interest in prostaglandin balance and fluid/protein transudation. This line of investigation was, of course, preliminary and may warrant further work.

-Blood Analysis:

Aseptically, blood was taken simultaneously as lymph from the right external jugular. Samples were routinely submitted for total protein after being cooled, and for cultures as well.

-Tissue Analysis:

A lung tissue sample was obtained by aseptic percutaneous needle biopsy of the left lower lobe. The specimen was submitted for qualitative culture analysis. Bilateral mini-thoracotomies were performed again in the first 8 experiments, and quantitative culture results of the bilateral tissue samples consistantly revealed no growth for the specific bacteria to be instilled. Therefore, the single needle biopsy obtained the same information with less trauma to the thorax. Post-biopsy chest X-ray did not reveal any significant pneumothoraces. Tissue specimens at 24 hours were obtained through bilateral thoracotomies before sacrifice of the animal, and were quantitatively cultured. Samples were also examined histologically using an H and E stain.

After baseline procedures are completed, the animal is transferred to a modified Mark IV respirator for aerosolized infusion of 10 cc of 10^8 bacterium (see Fig. II). The jet nebulizer atomizes the solution, which is delivered at a pressure setting of 30-35 and under 5 cm of H20 PEEP. The inoculation requires less than an hour; after which the animal is removed from the respirator if able or supported temporarily on the volume respirator. The exhalation water was sterilized

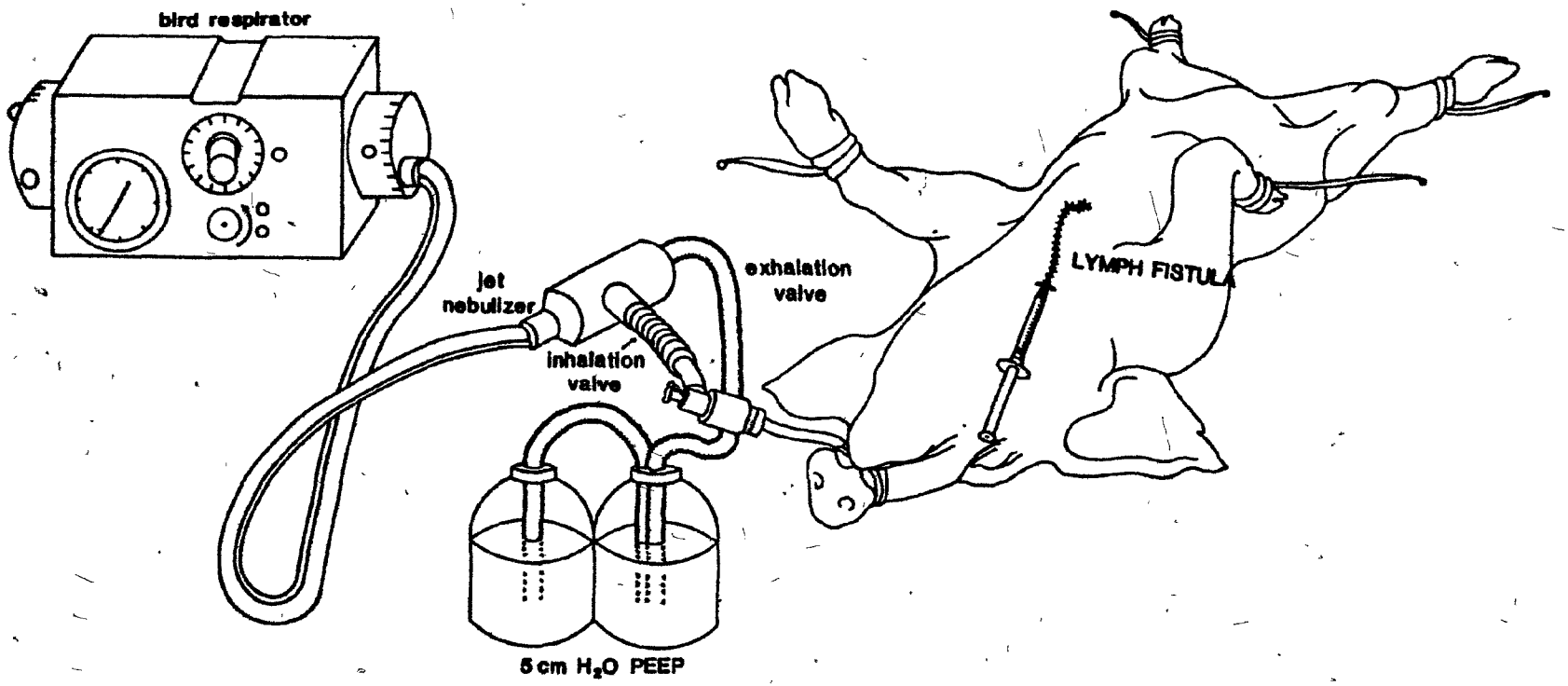

Figure II: Model preparation completed, with modified Bird Respirator infusing bacteria under 5 cm PEEP. Lymph fistula is completed.

with the addition of an antiseptic solution to reduce the number of viable exhaled bacteria.

In 3 final experiments, the AM function was examined with collapse only without infection.

-Collapse:

Collapse of the lung was achieved by 2 techniques for the first 8 experiments, after which one was used exclusively. The first and primary technique involves the introduction of 2 Swan-Ganz balloon catheters in tandem endobronchially into the right main bronchus or into the right apical bronchus if the animal weighed less than 20 kg (Figure III). The balloons were inflated with quick-dry epoxy to prevent any deflation over the 24 hour period. Catheters were modified to exit the trachea in the neck to avoid damage by mastication.

The second technique to collapse the right lung was by the introduction of air under 40 mm Hg to create a right pneumothorax (Figure IV). A #18 gauge needle was placed percutaneously with a pressure bulb to introduce the air. Collapse was conducted under fluoroscopic control as well, and post-chest X-rays were taken to confirm the collapse.

Animals were removed from any ventilatory assistance when awake and returned to an overnight holding cage with a low dose morphine drip (10 mg in 1 liter) running.

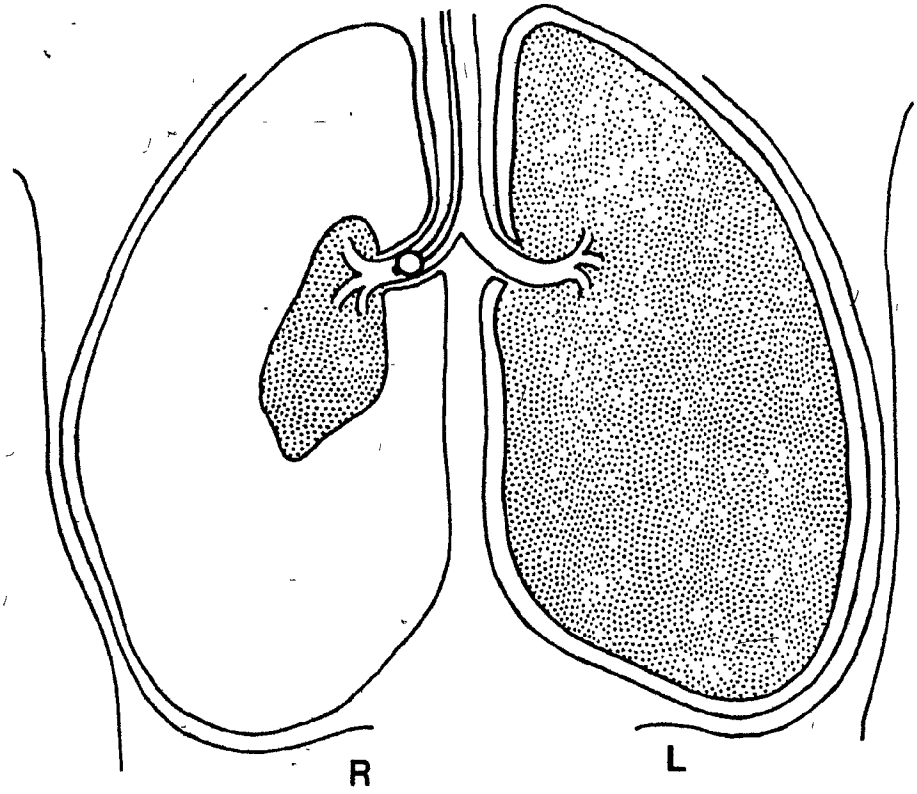

Figure III: Schematic of lung collapse using endobronchial balloon catheter.

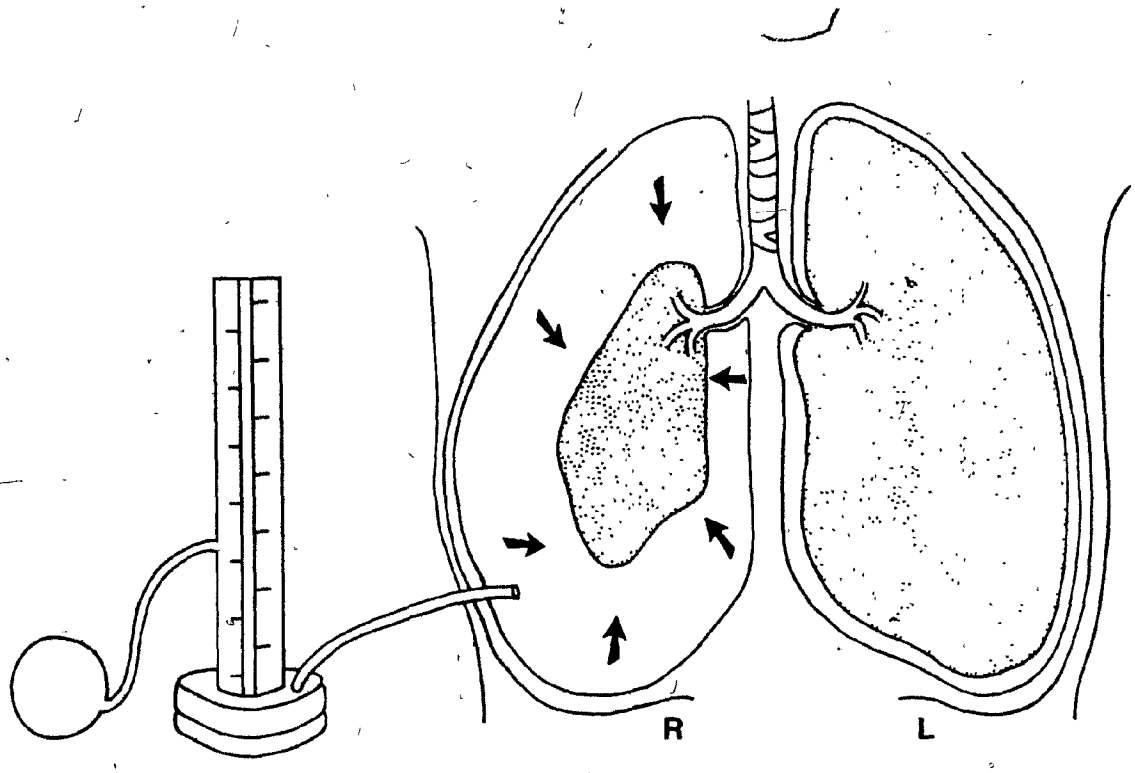

Figure IV: Illustration of right pneumothorax by instilling air under 40-45 mm Hg.

At 24 hours, the animal was returned to the operating theater and a repeat of the baseline measurements and tissue/fluid samples were taken prior to sacrifice.

-Microbiology:

Four separate bacteria were utilized in our study, the first 3 discarded over the initial 6 experiments. The organism utilized in the last 16 experiments was Streptococcus Pneumoniae (or Pneumococcus) Type III. The other bacteria examined were in order: Pasteurella for the first 2, endogenous E Coli in the next 2, and Staphylococcus Aureus the next 2. Pneumococcus Type I was used in 1 experiment. All bacteria were prepared overnight in TSB culture medium to a concentration that was verified of 10^8 organisms per ml.

The bacterial quantitation technique utilized for lung tissue is as follows:

-Bacterial Quantitation:

1- Containers are preweighed and hold a known weight of TSB culture medium.
2- Lung tissue is added, and the container is reweighed.
3- The tissue is ground using a "Polytron" homogenizer, after which
4- the suspension is diluted according to a viable count method as established by Miles and Mizron (see below).
5- A viable count is performed and the number of organisms per gram weight of tissue is calculated.

-Viable Count:

1- Tenfold dilutions of 10^{-1} to 10^{-5} or (0.5 ml plus 4.5 ml of TSB) are made.

2- Three blood agar plates are dried for 1 hour at 37°C.

3- The plates are divided into 6 sections, and 1 drop (1/50 ml) of each dilution is placed onto each segment (0 to 10^{-5}). The specimens are tested in triplicate.

4- Specimens are then incubated at 37°C for 18 hours and segments are read with 20 to 200 counts/segment. The count is averaged from 3 plates.

-Alveolar Macrophage Tests:

The lavage specimens are transferred immediately to the lab in chilled polypropylene tubes, and the AM are collected in the following procedures. A cell count and differential is performed as the initial step in the process.

-Technique for total and differential cell count:

1- For total cell count use hemocytometer and express the count as cells per ml.

2- For differential cell count, 0.1 ml of cell suspension was cytocentrifuged at 1,500 RPMs for 5 minutes and allowed to dry. It was then stained with Diff-quik (Harleco), a Wright's stain.

3- One hundred cells were counted under oil immersion

and the percentage of macrophages was made.

4- The cell suspension was centrifuged, noting the volume at 1,500 RPMs for 10 minutes and the pellet was resuspended in the medium with 10% fetal calf serum in order to have a final concentration of 2 x 10^6 macrophages per cc.

5- Cell suspensions are kept on ice at all times.

-Chemotaxis:

Chemotactic factors were experimented with throughout the study to obtain the optimum responses. We utilized mainly, 8 experiments, endotoxin-activated serum, and in 4 experiments the tripeptides - n-formly methionyl alanine and n-formly methionyl phenylalanine.

-Chemotaxis Assay:

1- All assays are run in triplicate using RPMI medium 1640 and 20 millimolars of Hepes Buffer as diluent (lower chamber of the Blindwell chamber).

2- The lower chambers were prepared using 200 ml with
   a) control chambers with medium alone and
   b) to the lower chambers, each dilution of the chemotactic factor in the medium was added.

3- A membrane or filter with pore size 5 microns made by Neuroprobe Corp. (Bethseda, Md.) is then applied to the chamber and the chamber is incubated at 39°C with C02.

4- 200 microliters of 2 x 10^6 macrophages per ml suspension is pipetted into the Dulbecco's medium

and 10% fetal calf serum, thus providing 500,000 macrophages in the upper chamber.

5- This solution is then incubated at 37°C with C02 for 4 hours. After incubation, the membranes are removed carefully and placed upside down on a slide, to dry and stain them with Diff-quik. Under immersion oil, the macrophages per 20 fields were counted.

-Phagocytosis:

As in the chemotaxis technique, we made modifications during the study. In particular, we utilized a tube system made of polypropylene (Falcan 12 x 75 mm cap), in place of the well system; that will be discussed in a later section. In the initial 3 experiments, we utilized the latex bead technique, but quickly discarded it in favor of the less subjective, more accurate radio-isotope method.

-Phagocytosis Assay:

1- 500,000 macrophages in 1 ml Dulbecco's medium and 10% fetal calf serum are added to 16 mm culture wells (Tissue Culture Cluster 24, Costar) and incubated 2 to 3 hours at 37°C with 5% C02 in moist air.

2- The macrophage mono-layer is washed with 1 ml medium and 10% fetal calf serum 2 to 3 times to remove non-adherent cells.

3- 0.5 ml of chromium 51-EAIgG in medium of 10% fetal calf serum is then added and the mixture incubated

1 hour at 37°C with 5% C02 in moist air, then washed 3 to 4 times with VBS-gel buffer. The pellet is resuspended in 25 ml of medium and 10% fetal calf serum is added.

4- The specimen is washed with VBS-gel buffer (isotonic veronal buffered saline with 0.1% gelatin, 0.001 molar Mg and 0.0001 molar Ca), to which is added 0.5 ml of lysing buffer for 60 seconds until all non-phagocytized erythrocytes Ea are lysed. The macrophage mono-layer is washed twice with 1 ml of VBS-gel.

5- Finally, 0.6 ml of sodium dodecyl sulphate 0.5% is added and a 0.5 ml sample is counted in the gamma-counter, the total count being 0.5 ml of 51-Cr-EAIgG and a percentage of phagocytosis.

The chromium tagged mixture is made by mixing 1 ml erythrocyte of concentration 1 x 10^9 cells per ml with 0.5 ml of rabbit anti-Forsmann antisera and 0.1 ml of 51-Cr.

-Intracellular Bactericidal Assay:

For 5 of these tests, we used the in vivo culture medium containing Pneumococcus Type III as a phagocytizable bacteria. In 3 other experiments, we utilized in vitro exposure to both E Coli and Staphylcoccus Aureus. Both techniques achieved satisfactory results.

-Technique:

1- Following a suitable culture period, the mono-layer

is rinsed and transferred to a medium containing a bacterial specific antibody inactivating the extracellular organism, but not penetrating the phagocyte membranes.

2- The mono-layer is then washed several times with normal saline to remove all non-adherent cells.

3- Culture was then made of this cleansed mono-layer.

4- The mono-layer was exposed to hypotonic sterile water with the freeze-thaw technique using dry ice for 45 seconds, to lyse the macrophages to release the intracellular organisms.

5- This was then cultured and the comparison between the initial and the second cultures revealed the percentage of viable organisms remaining, or in other words, the bactericidal capability of that macrophage system.

-Electron Microscopy:

The centrifuged pellet from the lavage aspirate is fixed in glutaraldehyde for 2 hours and rinsed in phosphate buffer. Specimen is then placed in osmium tetroxide for I hour with graded acetone 25%, 75%, 95% and absolute. Following this, it is embedded with 50-50 acetone and epon embedding media, and the thin sections are then stained with uranyl acetate and lead citrate for 10 minutes each.

RESULTS

> If an experiment turns out precisely as predicted, this can be very nice, but it is only a great event if at the same time it is a surprise. You can measure the quality of the work by the intensity of astonishment. This surprise can be because it did turn out as predicted (in some lines of research, 1 percent is accepted as a high yield), or it can be confounded because the prediction was wrong and something totally expected turned up, changing the look of the problem and requiring a new kind of protocol. Either way, you win (172).

-Model:

Aerosolized bacteria with PEEP evenly distributed the inoculating bacteria throughout all lung fields, particular right and left. This was established by tissue cultures.

Collapse of the lung was complete using the modified balloon catheter technique. Figures V-VIII are representative photomicrographs of the changes the collapse (and infection) incurred with comparison to the non-collapsed site. These examples are taken from the same animal who underwent a 24 hour experiment.

Figure V is lung tissue at baseline, obtained through a needle biopsy. Figure VI is taken from the non-collapsed site at 24 hours. Although it shows alveoli septal wall thickening secondary to inflammation, the architecture is easily recognizable and a moderate number of alveoli are ventilated.

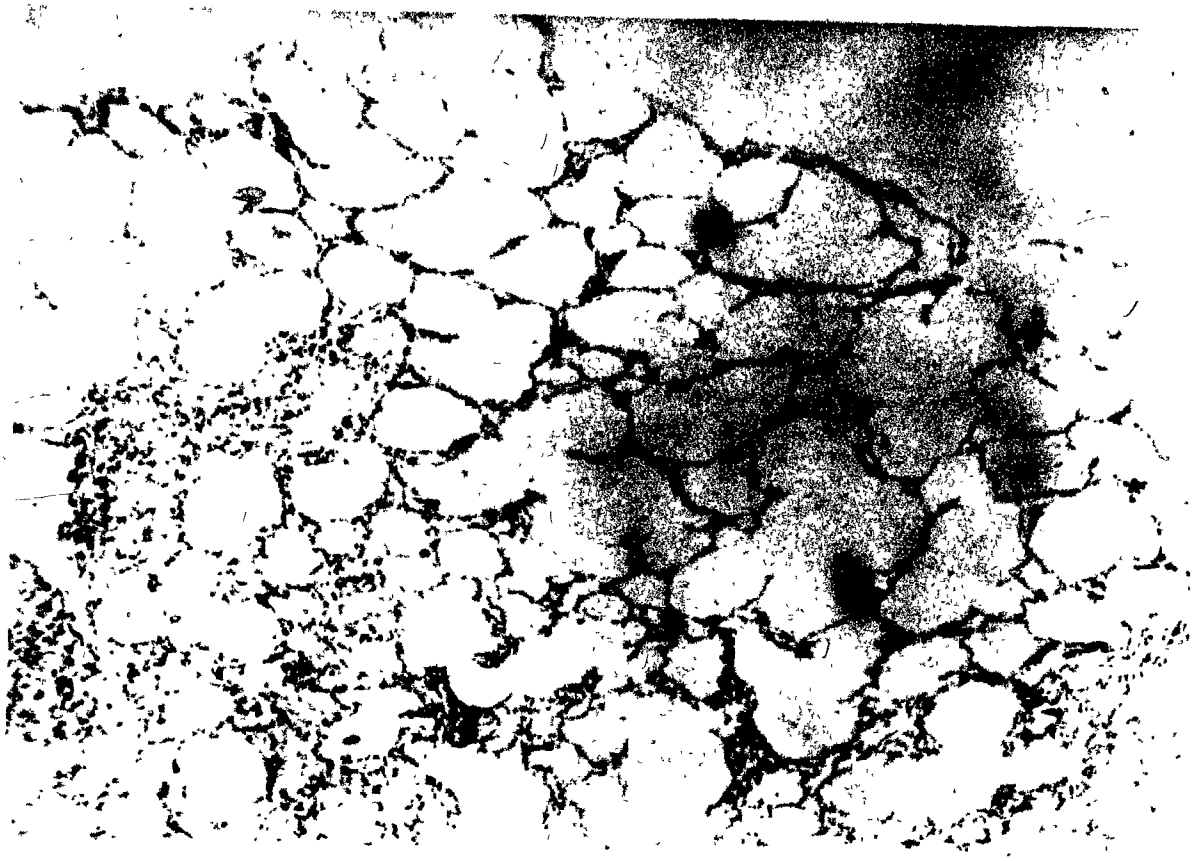

Figure V: Normal baseline lung tissue taken with needle biopsy.

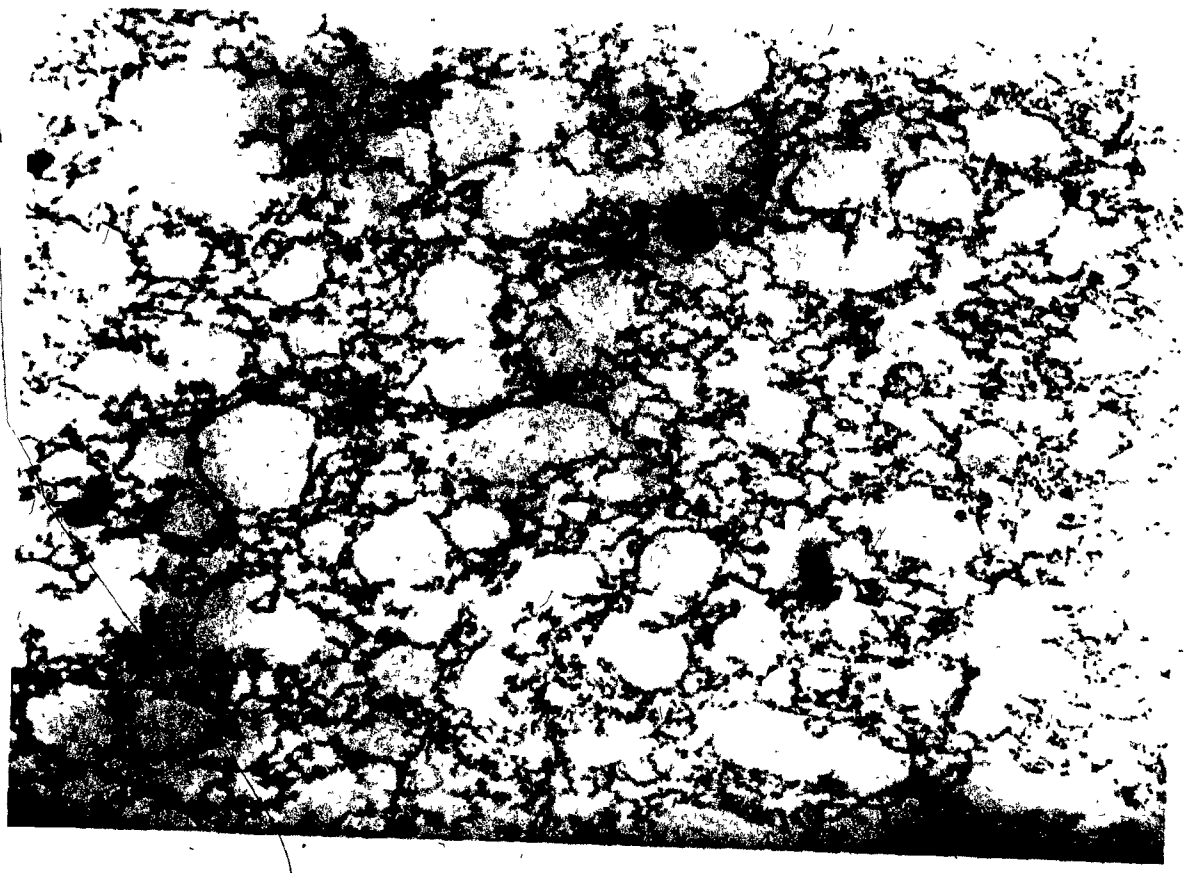

Figure VI: Control, non-collapsed lung at 24 hours after bacterial inoculation.

In contrast is Figure VII which is taken from the lung collapsed by endobronchial obstruction at 24 hours. This demonstrates almost total collapse, particularly the close-up of the bronchioles in Figure VIII are collapsed and commensurate with a very severe atelectasis. The cellular infiltrate in the collapsed lung at 24 hours is prominently polymorphonuclear and consistent with an early frank pneumonia.

The second manner of collapse by creating a pneumothorax was effective at achieving similar results to above. Figure IX is an example of the chest X-ray of an animal with a right pneumothorax, induced as described earlier. One can appreciate good collapse of the lung parenchyma, however, this also demonstrates a marked shift of the cardiac shadow into the left chest under a tension mechanism. The shift is particularly prominent in the pig with loose mediastinal attachments, and resulted in severe hemodynamic compromise. In order to cause sufficient parenchymal collapse, at least 40 mm Hg pressure had to be placed in the right hemithorax, causing too great a shift. Therefore, this technique was abandoned after experimentation in the early animal models.

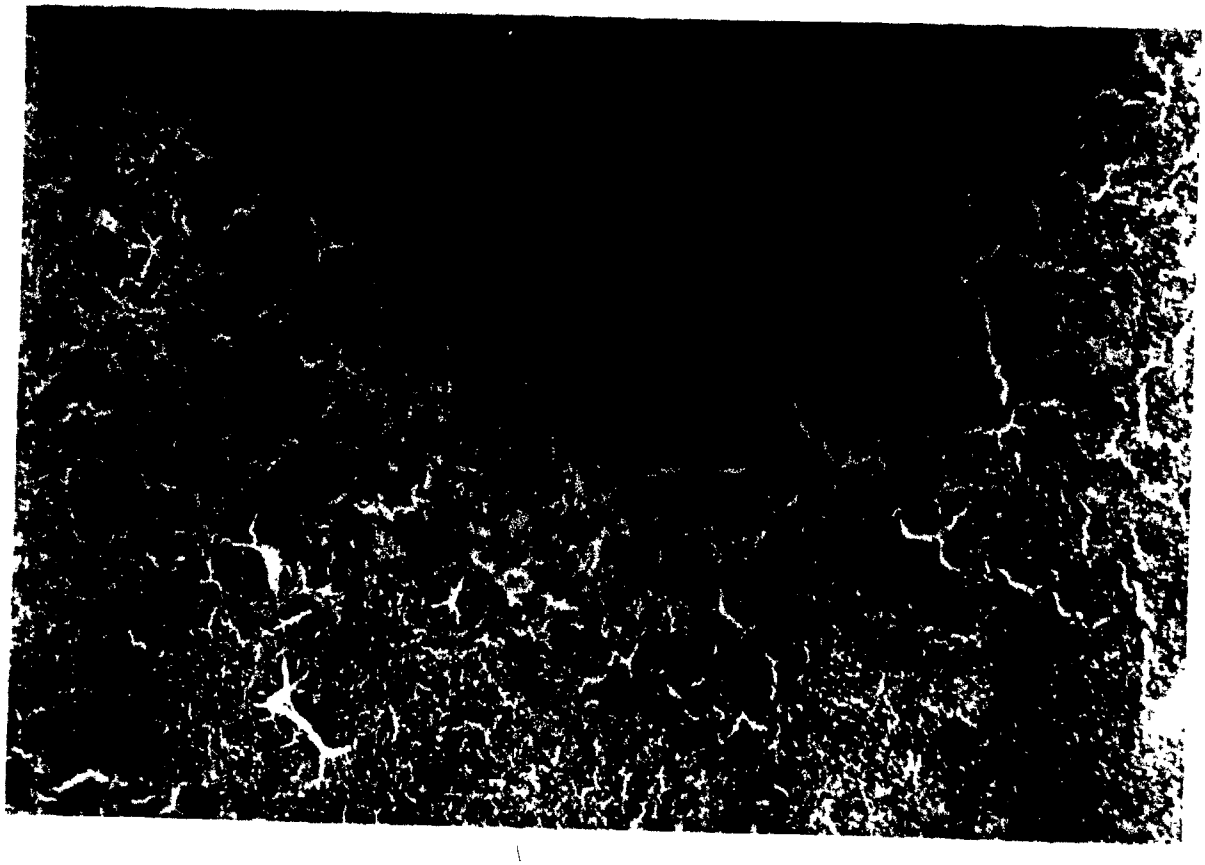

Figure VII: Lung tissue at 24 hours after bacteria instilled and lung collapsed by endobronchial balloon. Inflammatory cell content consistent with an early frank pneumonia.

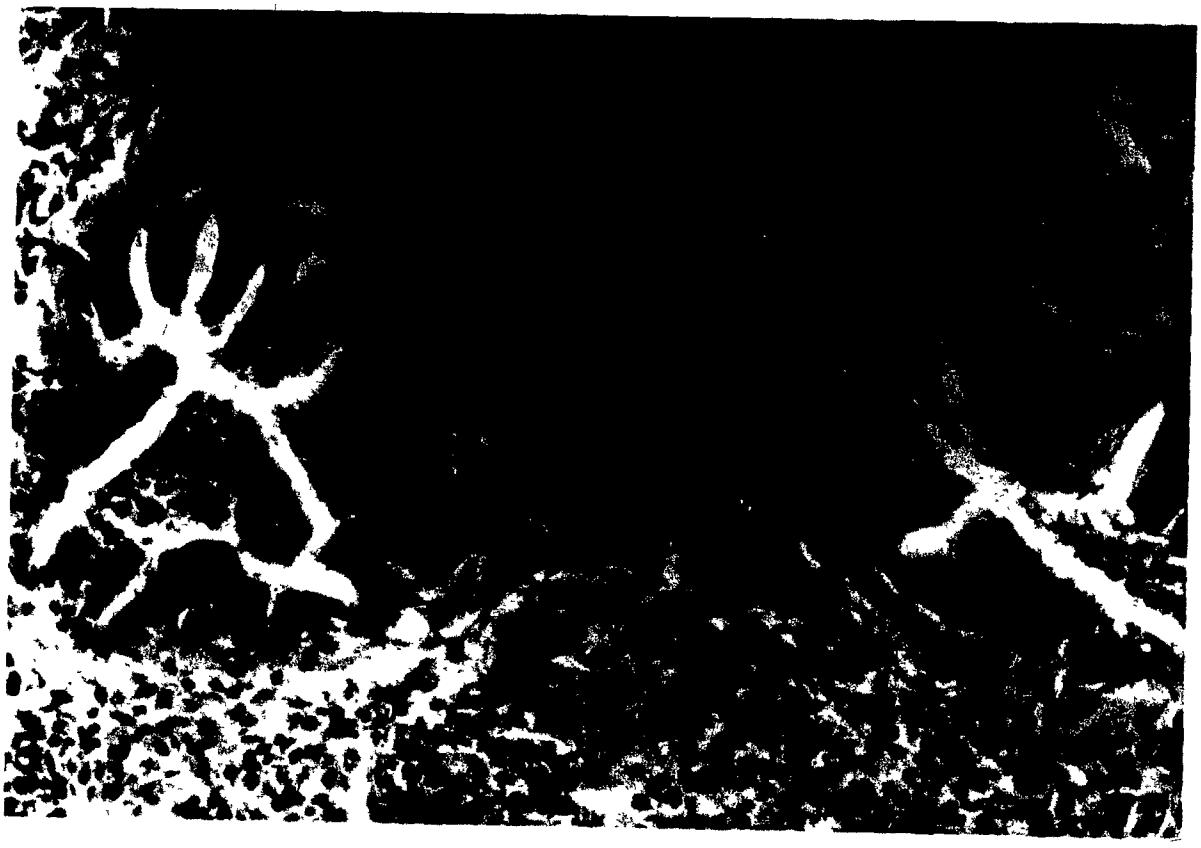

Figure VIII: Higher photomicrograph of collapsed, infected lung at 24 hours. Showing collapse of terminal bronchioles as well as alveoli, consistent with a severe atelectasis.

Figure IX: Chest X-ray of pig after creation of a right pneumothorax. Note marked shift of cardiac silhouette into left hemithorax.

MICROBIOLOGY:

-Bacterial Clearance:

Bacterial clearance in this case is the simultaneous comparison between the collapsed and the non-collapsed lung tissue of viable remaining bacterial inoculum, in this case Pneumococcus. The right lung collapse is constant throughout the experiment, as is the left lung as control. Bacterial growth is expressed in powers of ten.

-6 Hour:

Results from the 0-6 hour experiments (see Table 1) revealed that the collapsed lung had greater bacterial growth than the control, and therefore, a relatively decreased clearance function. Figure X expresses the results as a difference (in powers of 10) between collapsed and the non-collapsed here listed as control and representing the baseline. The 3 and 6 hour results are approaching significance with the Student's Paired t-test (2 tail), of 0.05 - .10.

TABLE 1 BACTERIAL CLEARANCE

	RIGHT LUNG (COLLAPSED)	LEFT LUNG (CONTROL)
1 H.	6.2×10^3	1.2×10^3
	1.7×10^6	0
	1×10^4	0
	2.5×10^4	0
	1.3×10^2	0
2 H.	1.7×10^4	2.3×10^4
	5×10^6	2.3×10^6
	3×10^4	6×10^3
	3.5×10^4	3×10^4
	1.3×10^2	5.2×10^2
3 H. *	3.3×10^4	6.3×10^3
	9.5×10^7	$3,7 \times 10^3$
	1.2×10^6	1.2×10^2
	1.5×10^6	7×10^2
	3.4×10^2	1.7×10^2
4 H.	8×10^3	2.2×10^3
	6.3×10^5	1.7×10^7
	8×10^5	3×10^2
	1.5×10^4	4×10^5
	1.2×10^2	7.9×10^1
5 H.	5.1×10^2	7.6×10^3
	4×10^3	3.2×10^2
	5×10^6	$.1 \times 10^3$
	1.2×10^2	1.9×10^2
6 H. *	2.7×10^3	8.1×10^2
	3.2×10^7	1.2×10^6
	6×10^6	2×10^3
	2×10^6	2×10^4
	$1,7 \times 10^2$	2×10^1

* P 0.05 – 0.10

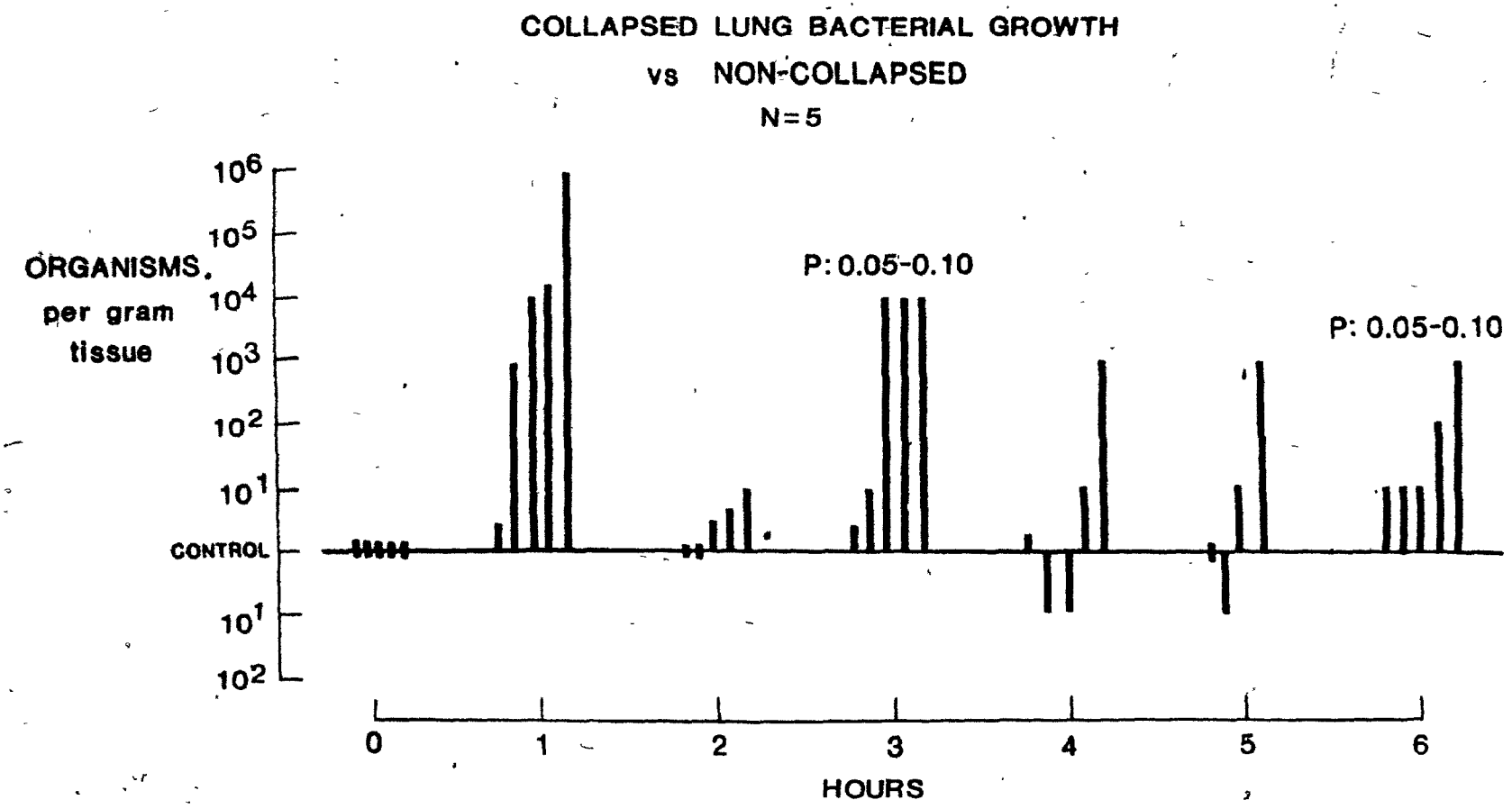

Figure X: Lung tissue bacterial clearance collapsed versus non-collapsed. Note decreased clearance in collapsed lung.

-24 Hour:

Bacterial clearance results from the overnight experiments are listed numerically in Table 2 and graphically in Figure XI where they are again expressed as the difference between the collapsed and non-collapsed or control lungs. There is almost uniform depression of bacterial clearance capacity in the collapsed lung. The results, however, were not statistically significant by Student's Paired t-test (2 tail).

TABLE 2: 24 HOUR BACTERIAL CLEARANCE

RIGHT LUNG (COLLAPSED)	LEFT LUNG (CONTROL)
4.7×10^5	1.2×10^4
3×10^4	2.2×10^4
1×10^5	1×10^2
0	0
8.5×10^6	1.3×10^4
1×10^1	0
6×10^3	1.5×10^4
0	0
2×10^3	2×10^2
6×10^4	3×10^3
1.1×10^3	0

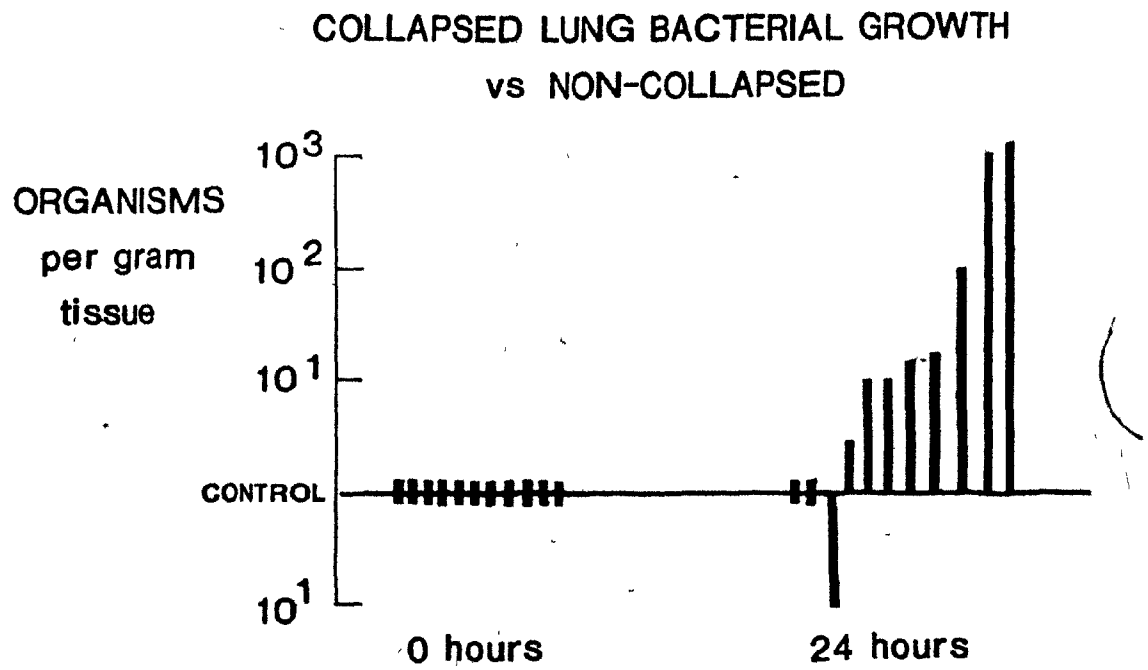

Figure XI: Lung tissue bacterial clearance at 24 hours comparing collapsed to the non-collapsed or control. Decreased clearance capability is evident in the collapsed lung.

-Exit Pathway:

The cultures taken of blood and lymph revealed that each was positive for the inoculating organism on 2 occasions. However, interestingly, they were not from simultaneous experiments. Blood was positive for Pasteurella once and Pneumococcus III on another occasion. The lymph cultures, on the other hand, both times grew out Pneumococcus Type III. Figure XIV is one of these lymph samples taken at 24 hours that had a positive Pneumococcal culture. A total of 9 lymphatic and 9 blood cultures were taken in the course of 9 separate experiments.

PULMONARY LYMPHATICS:

-Microbiology:

Culture results see Microbiology.

-Lymph Flow:

Flow as measured in the right lymphatic duct at baseline time period over 3 hours and at 24 hours over 3 hours are tabulated in Figure XII. Flow rates uniformly increased over the 24 hours from less than 2 ml/hour to as much as 25 ml/hour with infection and collapse. All infected lungs were inoculated with Pneumococcus Type III bacteria. Two experiments with collapse alone, without infection, (solid line) are tabulated and show generally less increase in flow rate as compared to the combination of infection/collapse. Finally, the animal pre-treated with indomethacin is included (alternating symbols), and it increased as much as the combination experiments. All increases were statistically significant with P value less than 0.01.

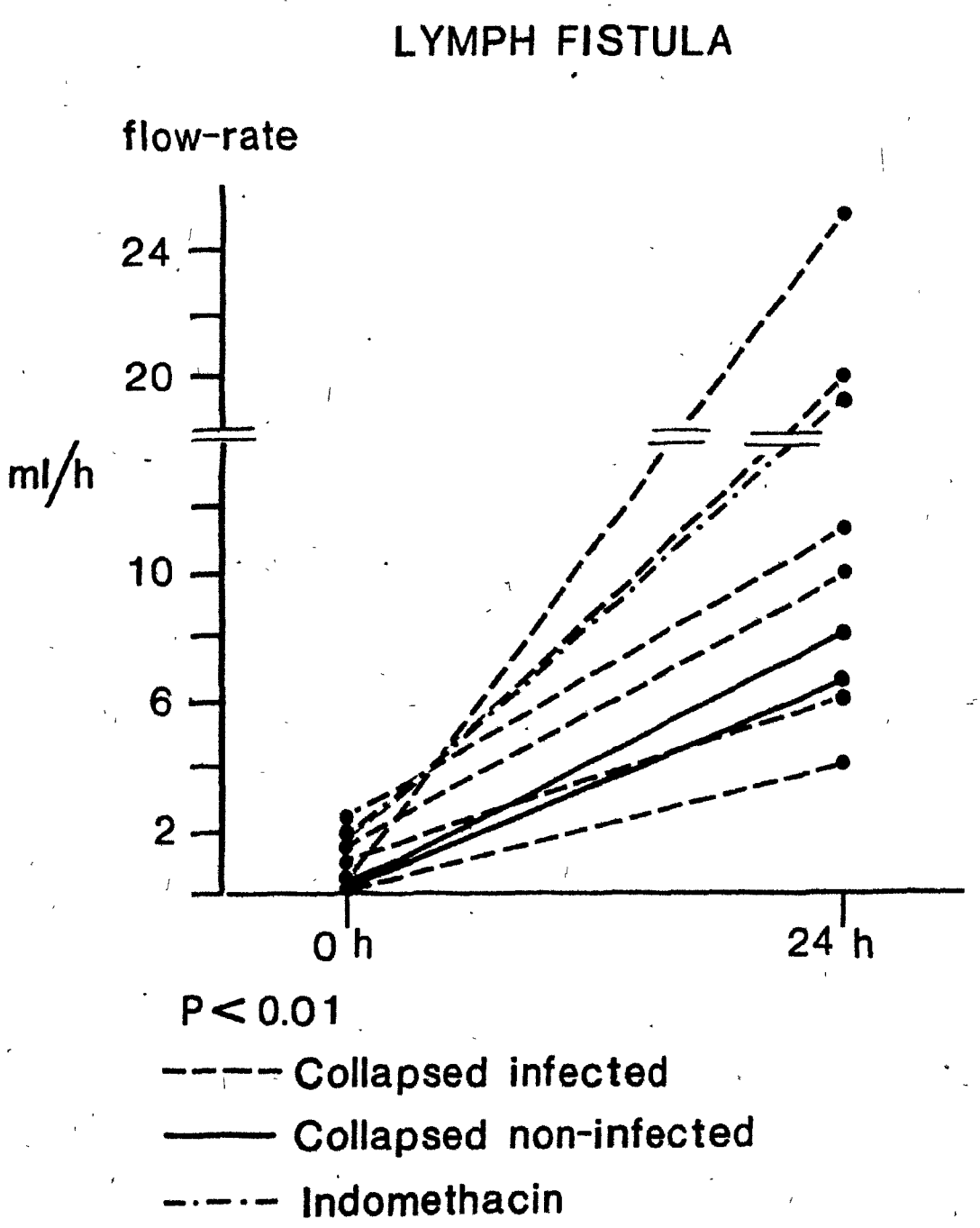

Figure XII: Lymph flow rate from right lymphatic duct cannulation measured at 0 and 24 hours.

-Lymph: Plasma Protein Ratio:

Figure XIII contains the results of the lymph: plasma protein measured at baseline and at 24 hours. It shows that the ratio consistently increased when the lung was infected, all with Pneumococcus Type III, and collapsed. P value was less than 0.01.

In marked contrast is the lymph: plasma protein decrease with either 24 hour pretreated with indomethacin or collapse alone without infection.

-Histology:

Figure XIV is the Geimsa stain of lymph taken from an infected, collapsed experiment at 24 hours. This lymphatic specimen likewise was cultured positive for the Pneumococcus Type III used as inoculum. The specimen is representative of the 24 hour lymph effluent in the other experiments and is remarkable for its mixed cellular content. There are a prominent number of RBC, here many are crenated due to artifact, in contrast to the usual large numbers of lymphocytes in normal efferent lymph. A number of AMs are prominent in the specimen (arrows), as are probable plasma cells or early AMs (open arrow).

ALVEOLAR MACROPHAGE FUNCTION:

-Morphology - Light Histology:

Figures XV-XVIII are photomicrographs of the bronchopulmonary lavage fluid taken from the same animal at baseline and at 24 hours from the collapsed and non-collapsed lung. Figure XV is a Wright's stain of the 0 hour specimen and contains normal appearing AM (open arrows), as well as a clump of plasma cells and lymphocytes (closed arrow).

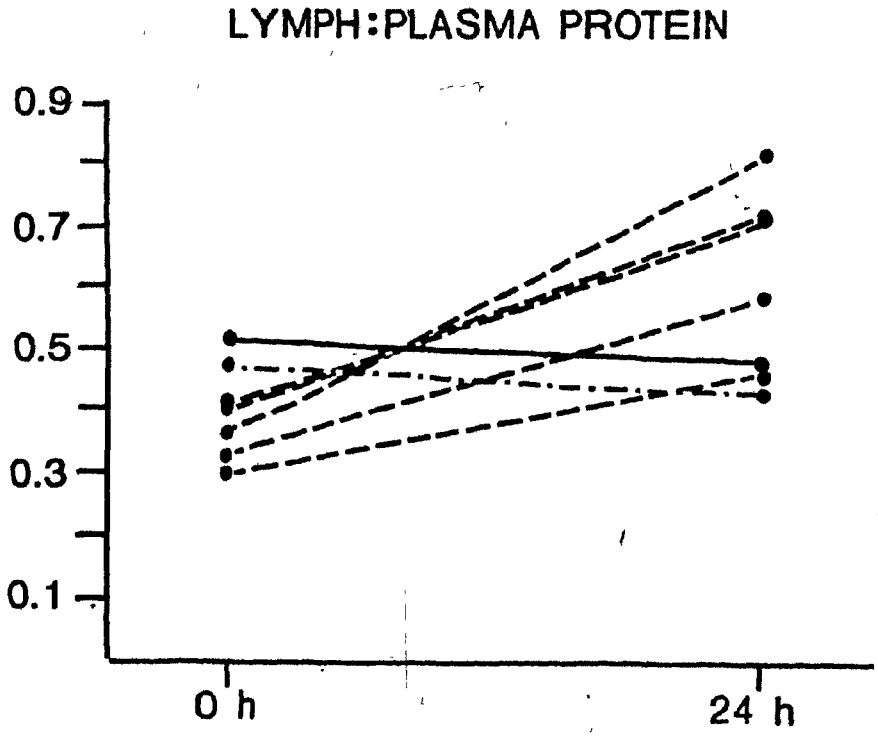

Figure XIII: The lymph to plasma total protein ratio measured at 0 and 24 hours.

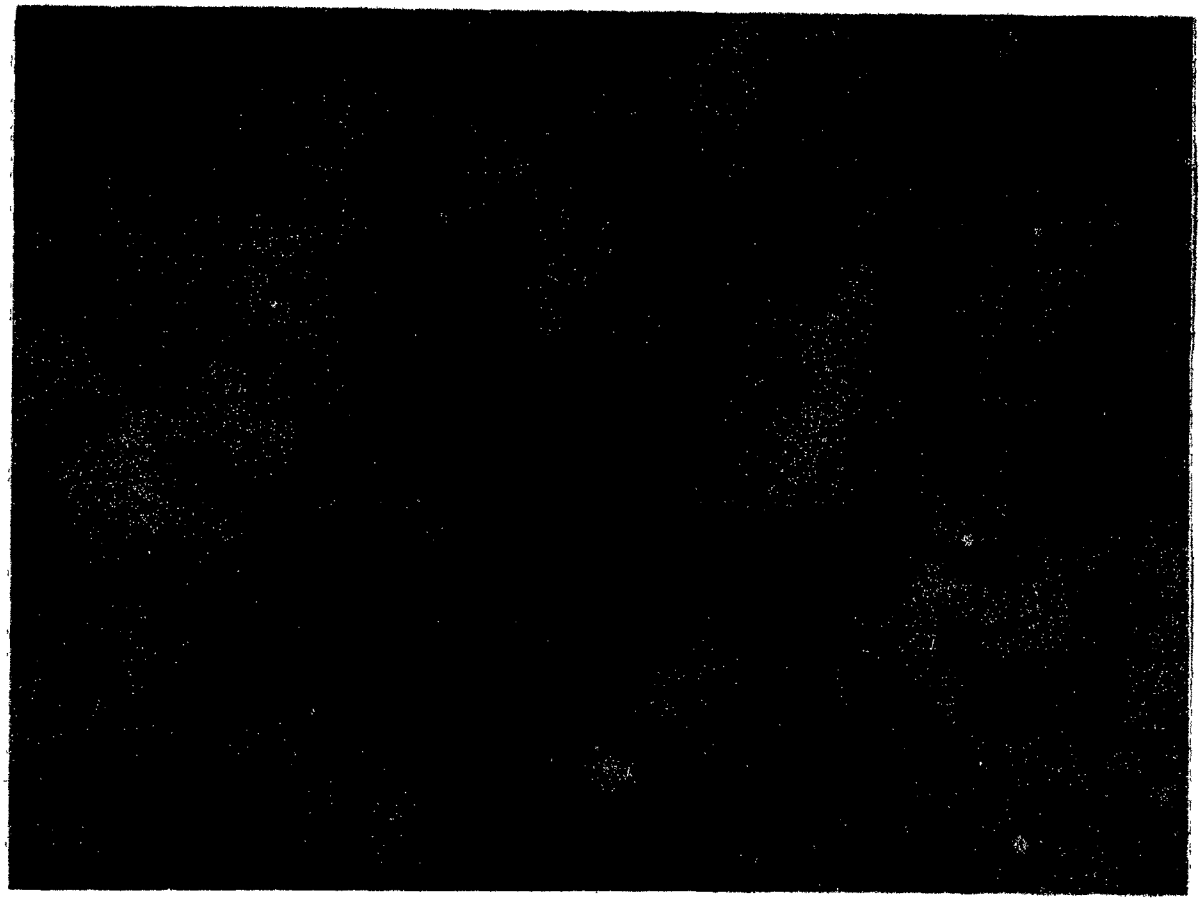

Figure XIV: Specimen 24 hour lymph from collapsed, infected lung. Note prominent background of RBCs, and as well AMs (closed arrows) and plasma or early AMs (open arrow).

COLOURED PICTURE

Figure XV: Baseline bronchopulmonary lavage specimen. Open arrows are AMs and the closed is a group of plasma cells and lymphocytes.

COLOURED PICTURE

Figure XVI is a photomicrograph of the lavage specimen taken from the control infected (non-collapsed) lung at 24 hours. The bacterial inoculum was Pneumococcus Type III. There is a distinct lack of B-cell response in this specimen as denoted by the relative absence of polymorphonuclear cells in comparison to Figure XVII from the collapsed lung. The control AMs appear to be normal in cytohistologic morphology.

Figure XVI: Bronchopulmonary lavage from infected control, non-collapsed lung at 24 hours.

COLOURED PICTURE

Figure XVII is a photomicrograph of the bronchopulmonary lavage at 24 hours from the collapsed infected lung, from the same animal as in preceding. Specifically, the specimen contains a prominent infiltration of polymorphonuclear cells. The AMs include numerous cytoplasmic vacuolations, but by light histology do not appear to be abnormal and to vary from the control. Figure XVIII is a clump of a group of AM from this specimen and evident is the normal cyto-histologic architecture we have described earlier (see Macrophages). Vacuolization in the cytoplasm is very marked in this photomicrograph.

Figure XVII: Bronchopulmonary lavage specimen from collapsed lung at 24 hours. Note prominent B-cell as well as T-cell responses to infection and collapse.

COLOURED PICTURE

Figure XVIII: Higher power photomicrograph of the 24 hour collapsed infected lavage. Note the numerous cytoplasmic vacuolizations (arrow) evident at this power.

-Ultrastructure - Electronmicroscopy:

The following series of electronmicrographs, figures XIX-XXII, are taken of the lavage samples from one animal at 0 hour non-infected, and 24 hour infected collapsed and non-collapsed. The specific areas and ultrastructure changes, particularly in the AM, are meant to be representative of the other experiments. EM was not routinely done, and in fact, was only performed in 2 experiments.

Figure XIX represents a baseline control at a power low enough to appreciate the interaction of the AM (open arrow) with the neutrophil (closed arrows). As we discussed in the review section on the AM, the clumping action by the granulocytes about the AM is at least in part mediated by chemotactic factors; and represents normal behaviour and function.

Baseline AM ultrastructure is examined at higher power in figure XX. Evident at this power are the elaborate and well developed organelle systems in the cytoplasm, particularly the mitochondria and free ribosomes. Absent is the cytoplasmic inclusion bodies as well as a prominent pseudopodia formation. An old phagosome is present in the upper portion of the AM.

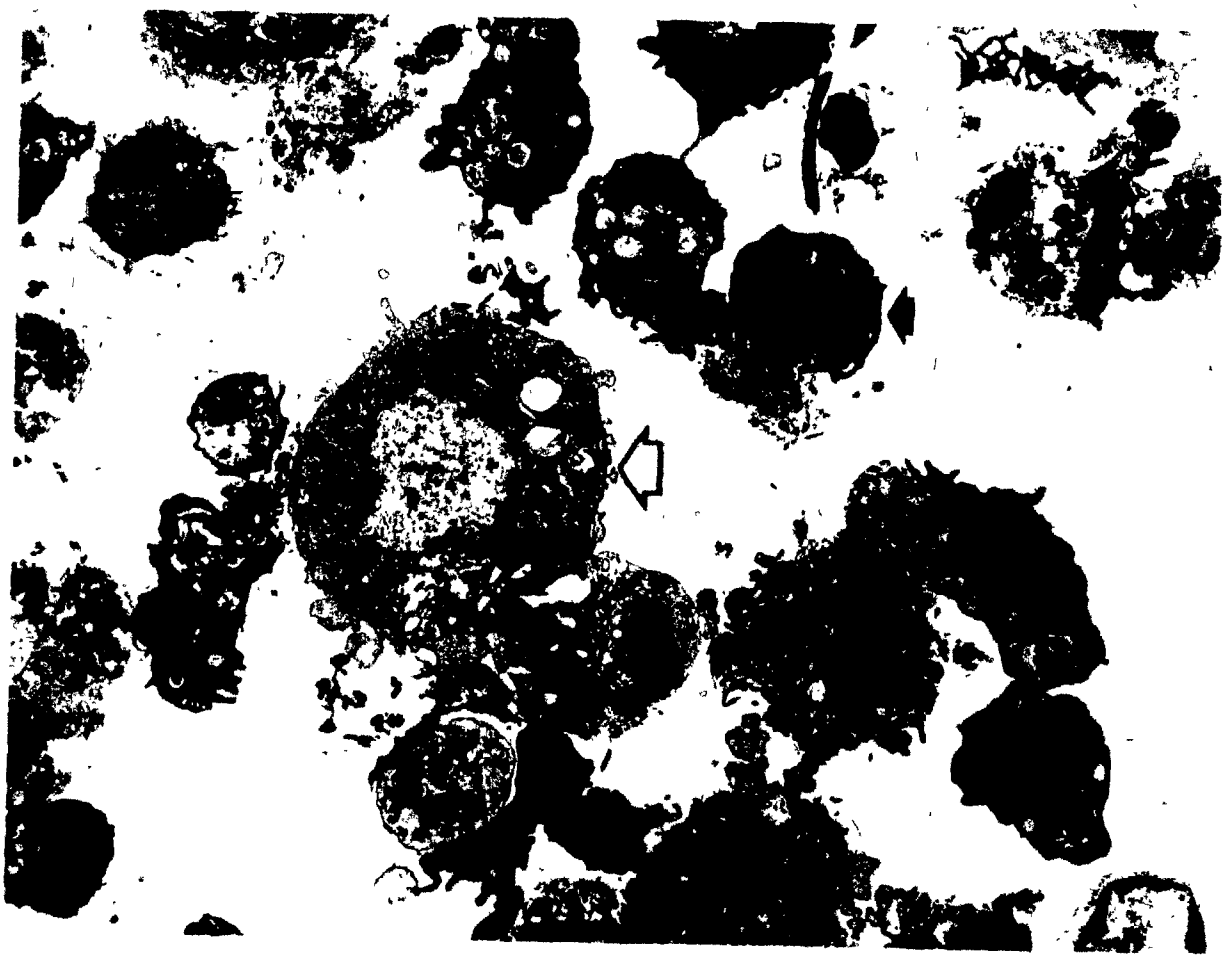

Figure XIX: Photoelectronmicrograph of the baseline lavage cellular contents, including the AM (open arrow) at the center, surrounded by granulocytes (dark arrows). This represents normal activity (Mag. x 4,000).

Figure XX: The AM at baseline contains well formed organelles in the cytoplasm, in particular the mitochondria (arrow) and the free ribosomes "dusting" throughout. Large arrow indicates an older phagosome. (Mag. x 11,600).

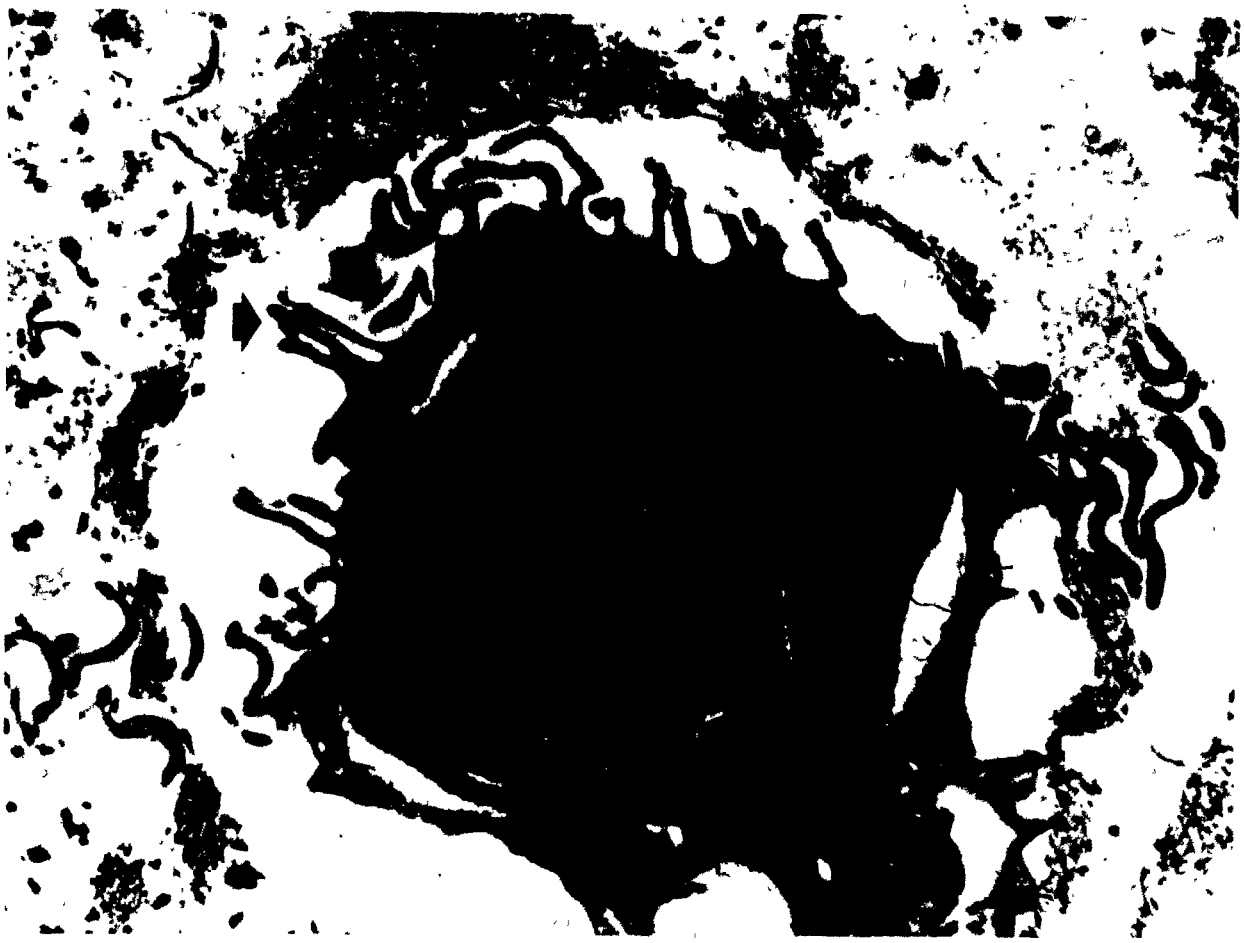

Figure XXI: AM ultrastructure from 24 hour infected control (non-collapsed) lung. Note large nucleus and well formed organelle systems. Pseudopodia is marked with an arrow. (Mag. x 11,600).

Figure XXI is an AM at 24 hours after infection from the non-collapsed or control lung. A prominent nucleolus is present as well as a well maintained organelle support system in the cytoplasm. Free ribosomes are noted throughout, as well as pseudopodia formation.

In contrast to the above, the AM from the infected collapsed lung at 24 hours contains a large amount of cytoplasmic or lysosomal inclusion bodies (arrow). In fact, endocytosis is in progress in the area to the right of the arrow. Although the cell still contains some mitochondria, their size and numbers are limited, and the cytoplasm is largely replaced by lysosomal enzyme inclusion, particularly in the area of endocytosis - pinocytosis.

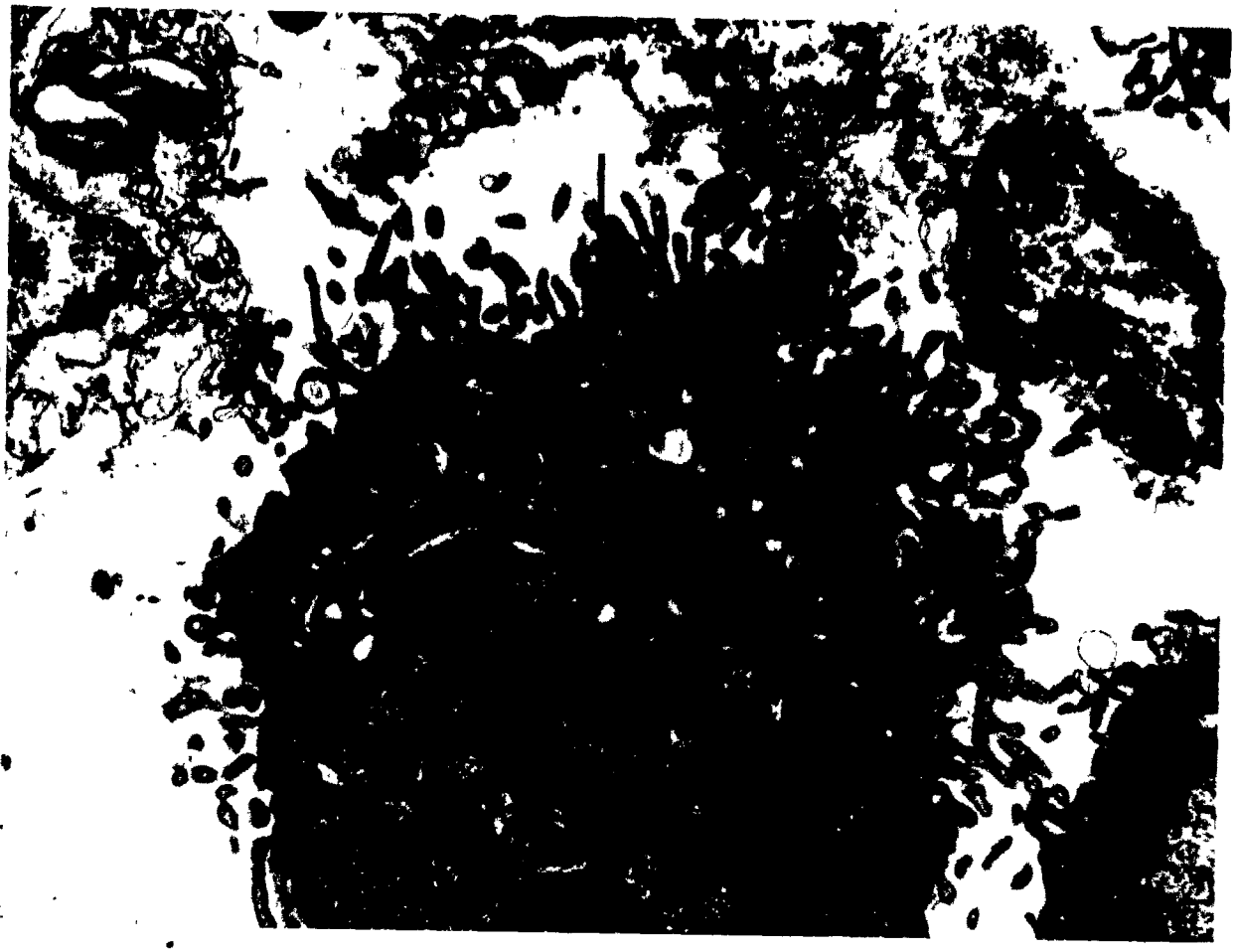

Figure XXII: AM ultrastructure from infected collapsed lung at 24 hours, has large amounts of lysosomal inclusions. Pseudopodia are also prominent; while the support organelles, i.e., the mitochondria. Golgi apparatus, are much less numerous and well preserved than in controls. (Mag. x 11,600).

AM CELL COUNT AND DIFFERENTIAL:

-AM Numbers:

Table 3 contains the total AM cell count per ml of lavage fluid at 6 hours, calculated by the technique previously mentioned. The results show that there is a drop in AM numbers in the collapsed infected relative to the control lung at this time. These findings are included in figure XXIII as well.

TABLE 3: AM CELL COUNT AT 0 AND 6 HOURS

The infected collapsed versus the control

(per ml lavage fluid)

0 Hour	6 Hours	
	Right (Collapsed)	Left (Non-collapsed)
6.2×10^6	3.9×10^5	2.1×10^6
8×10^5	3.5×10^5	1.0×10^6
5×10^6	1.4×10^6	5.1×10^6
6.2×10^6	3.9×10^5	6.8×10^5

(P 0.05 - 0.10)

The AM numbers for the 24 hour collapsed infected specimens are tabulated in table 4 and graphically illustrate in figure XXIII which is a composite of the 6 and 24 hour results.

TABLE 4: Number AMs - Infected collapsed versus non-collapsed lung at 24 hours.

(per ml)

Right (Collapsed)	Left (Non-collapsed)
3.5×10^6	1.0×10^6
8×10^6	4×10^6
1.8×10^7	4.7×10^6
3.1×10^7	2.5×10^7
1.1×10^7	1.0×10^7
1.8×10^7	2.9×10^7
9.6×10^5	1.8×10^6
2.3×10^7	1.3×10^7
1.7×10^6	5.7×10^4

(P 0.05 - 0.10)

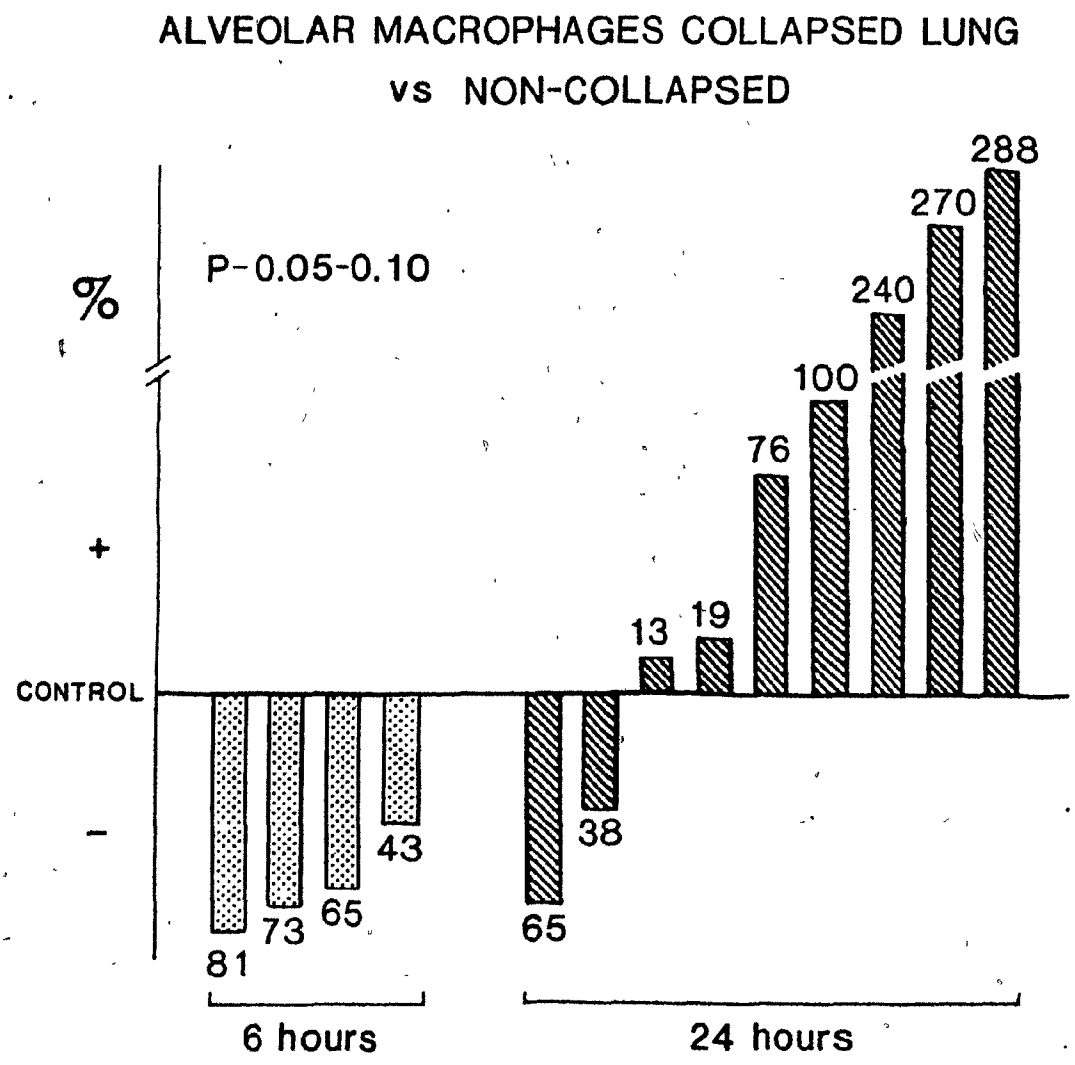

Figure XXIII: Alveolar macrophages numbers per ml lavage in infected collapsed versus non-collapsed at 6 and 24 hours. Note relative decrease at 6 hours and subsequent increase at 24 hours.

The AM numbers for the group of non-infected collapsed experiments are contained in Table 5. Of some interest is the fact that there seems to be no trend towards an increased number in the collapsed, in fact, 2 of the 3 are lower.

TABLE 5: AM NUMBERS FROM NON-INFECTED COLLAPSE VERSUS NON-COLLAPSED

(per ml)

Right (collapsed only)	Left (non-collapsed)
8.1×10^6	1.8×10^7
2.3×10^7	1.3×10^7
7.2×10^6	1.6×10^7

-Cell Differential Lavage Fluid:

Infected-Collapsed:

The percentage of AMs in the lavage specimen is calculated as noted in methods; and is assessed for the infected collapsed experiments (again Pneumococcus was used as inoculum). The results are tabulated in figure XXIV, where it demonstrates that over 24 hours, the AM % decreases in 5 of 7 from baseline, regardless of manipulation. Also evident is the fact that in 5 of 7, the AM % in the collapsed lung is less than that in the control, approaching significance at P 0.05 - 0.10.

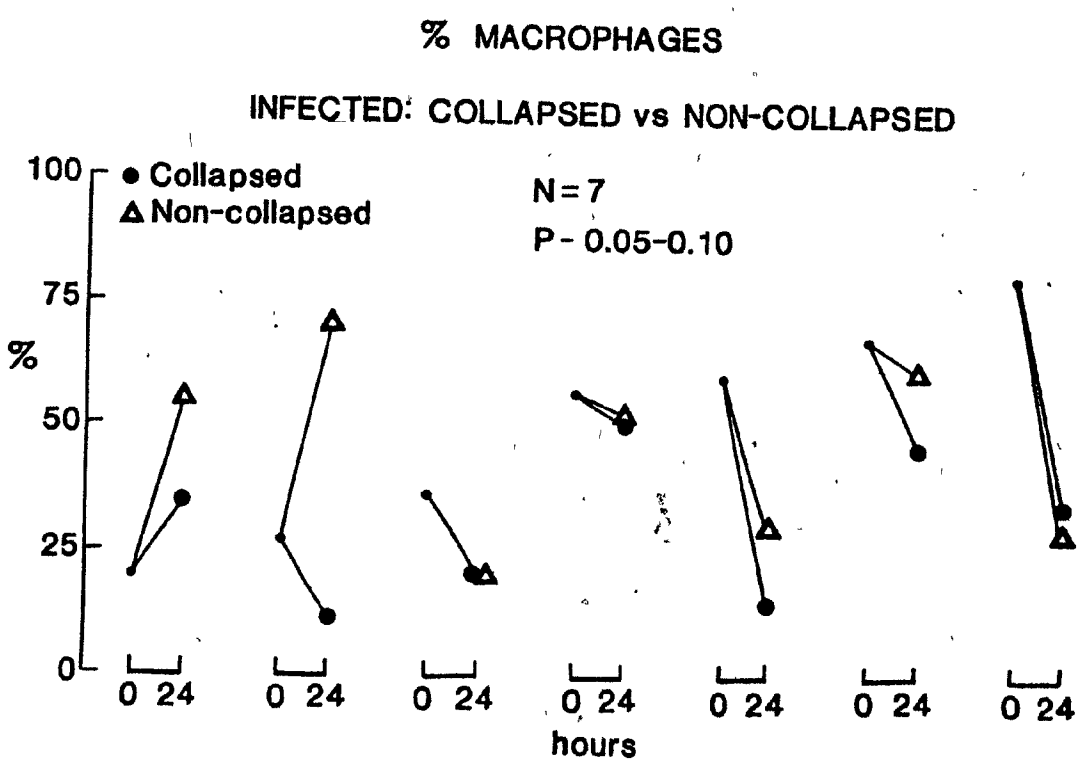

Figure XXIV: The percentage alveolar macrophages: Infected-collapsed versus non-collapsed over 24 hours.

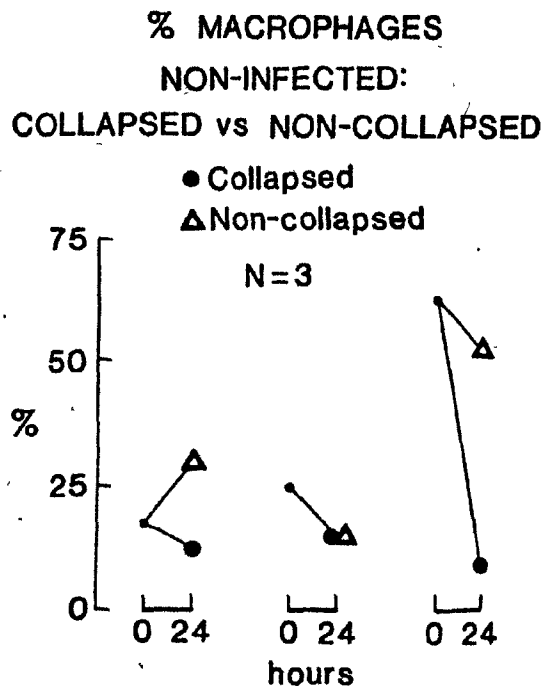

Figure XXV: % alveolar macrophages non-infected collapsed versus control.

-Non-infected-Collapsed:

Figure XXV contains the results from AM cell differential in lavage specimens in non-infected collapsed only experiments. This shows the same general trend with a decreased % of AMs in the collapsed alone compared to the control in 2 of 3. The results are included to list all results, but the small numbers in this series obviously cannot support any specific conclusions.

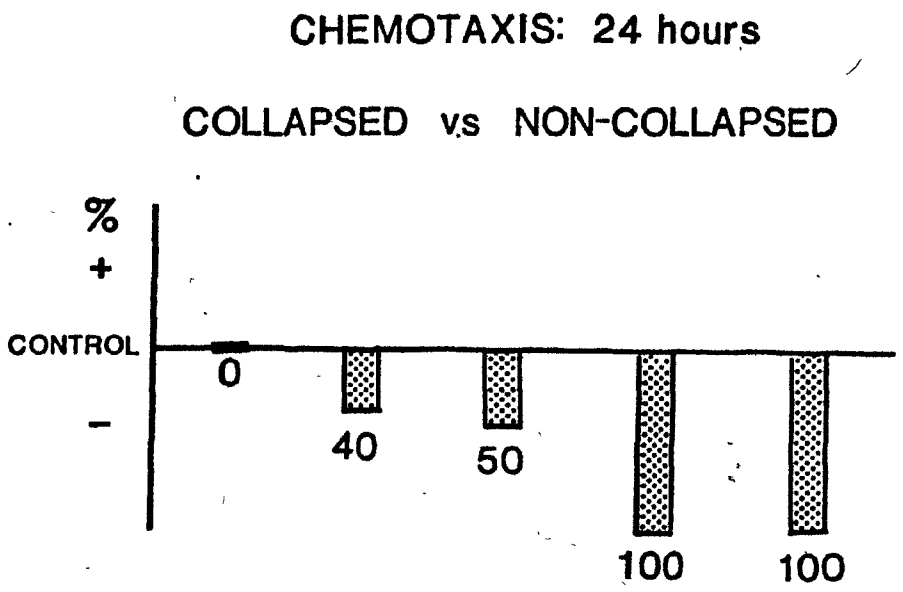

Figure XXVI: Chemotaxis results from AMs in infected collapsed lung versus the control at 24 hours, expressed as % difference.

ALVEOLAR MACROPHAGE FUNCTION:

-Chemotaxis:

AM chemotaxis results for the infected collapsed lung as compared by percentage to the control lung are demonstrated in figure XXVI. They indicate a depressed chemotaxis in the collapsed AMs. The chemotactic factor used was endotoxin-activated serum. The results are not statistically significant.

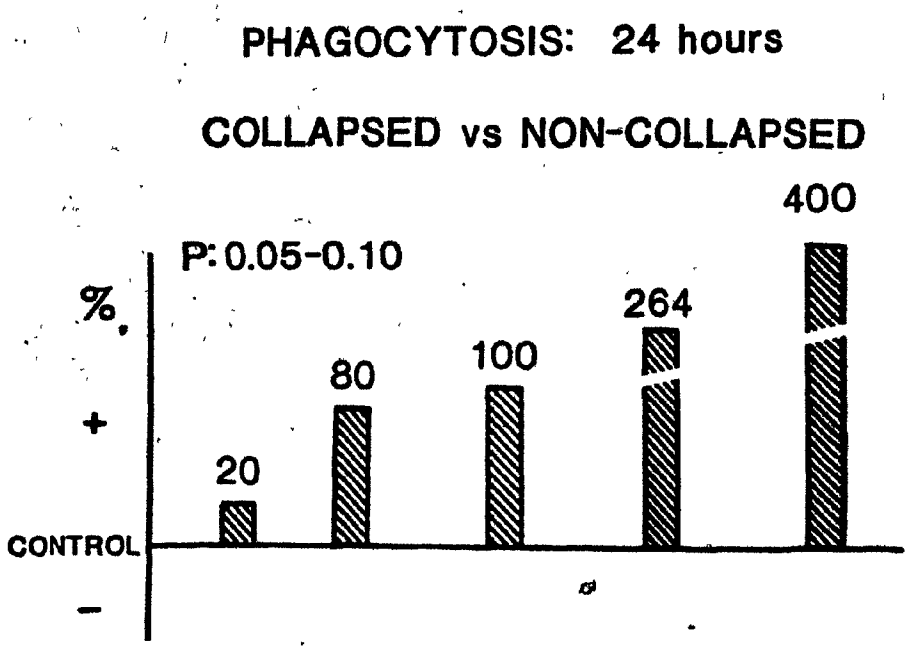

Figure XXVII: Phagocytosis for AMs from infected collapsed lung versus non-collapsed infected (control) at 24 hours as % difference.

PHAGOCYTOSIS:

-Infected-Collapsed:

Phagocytosis results are tabulated in figure XXVII where the AM phagocytosis from the infected collapsed lung is compared as a percentage to the control lung. Results approach statistical significance with P 0.05 - 0.10.

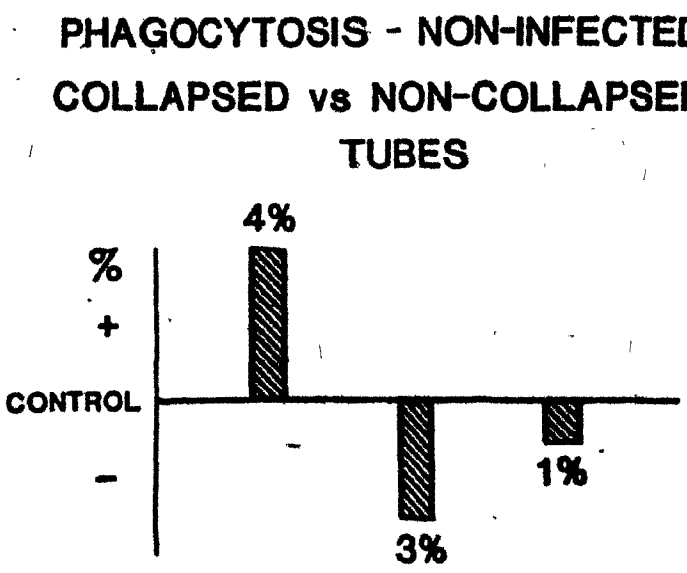

Figure XXVIII: Phagocytosis for AMs from non-infected collapsed lung versus non-collapsed at 24 hours.

-Non-infected collapsed:

Figure XXVIII shows that the AM phagocytosis did not seem effected, either positively or negatively, by collapse without infection. Indeed, the results between the 2 groups essentially did not vary.

-Well-Tube Controversy:

The results of utilizing both the usual 16 mm polystyrene culture well and a 12 x 75 mm polypropylene tube for simultaneous testing of AM phagocytosis is seen in figure XXIX. The polypropylene is a substance to which macrophages cannot adhere. The testing procedure which is essentially the same was performed on a single broncho-pulmonary lavage specimen in 10 separate experiments. Clearly, the use of the tube yielded significantly greater phagocytosis activity than the wells. $P < 0.001$.

-Bactericidal Assay:

Bactericidal assay was performed on 24 hour infected collapsed AM specimens and compared to the non-collapsed AMs. Figure XXX is the results tabulated as percentage difference. They demonstrate that the AMs from the collapsed infected side have, in fact, more bactericidal or intracellular killing activity than do those from the control side. Results are statistically significant at $P < 0.05$.

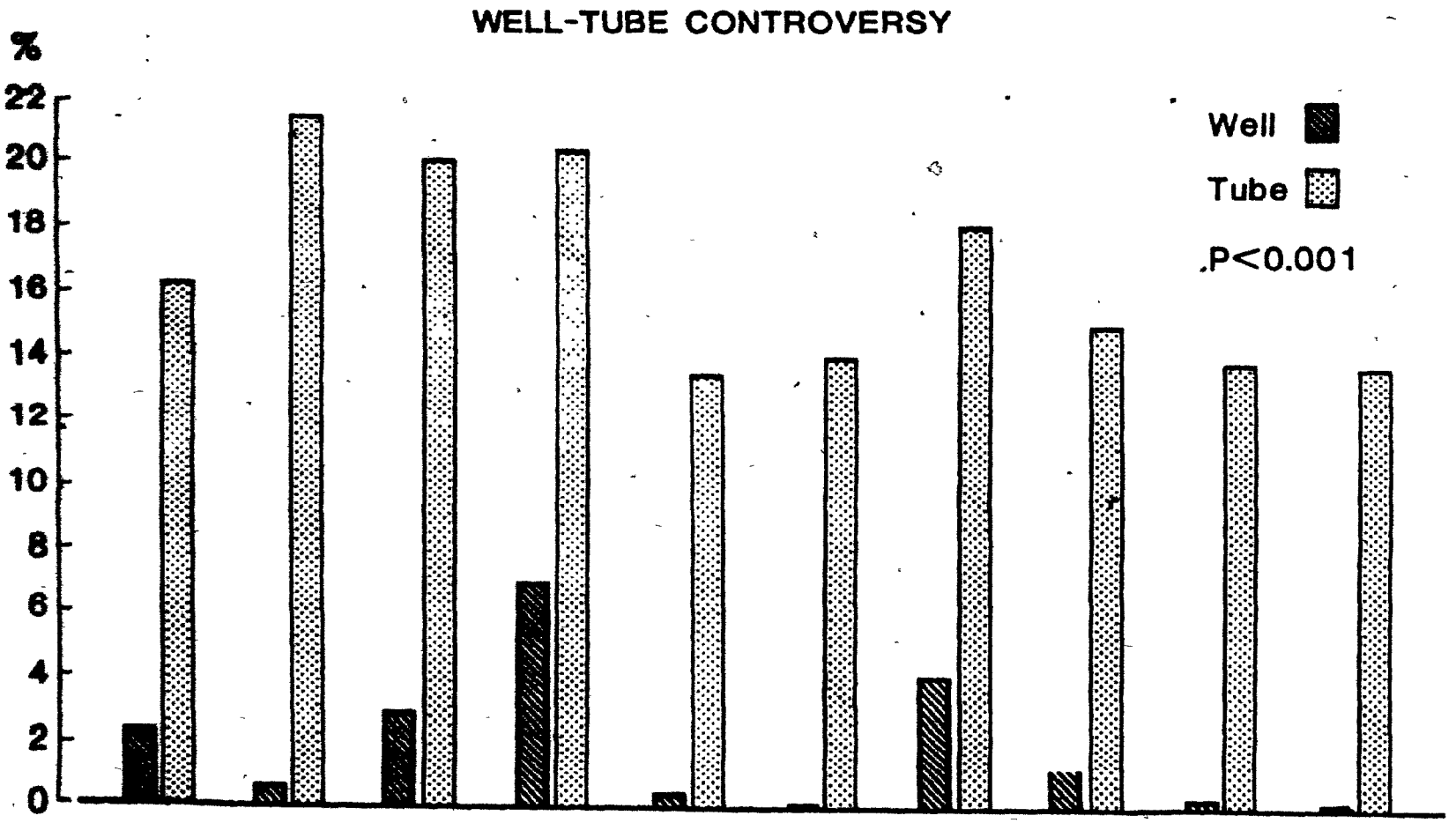

Figure XXIX: Phagocytosis performed with the same technique exchanging the routine polystyrene well, with a polypropylene tube. Specimens were from the 24 hour infected collapsed lungs.

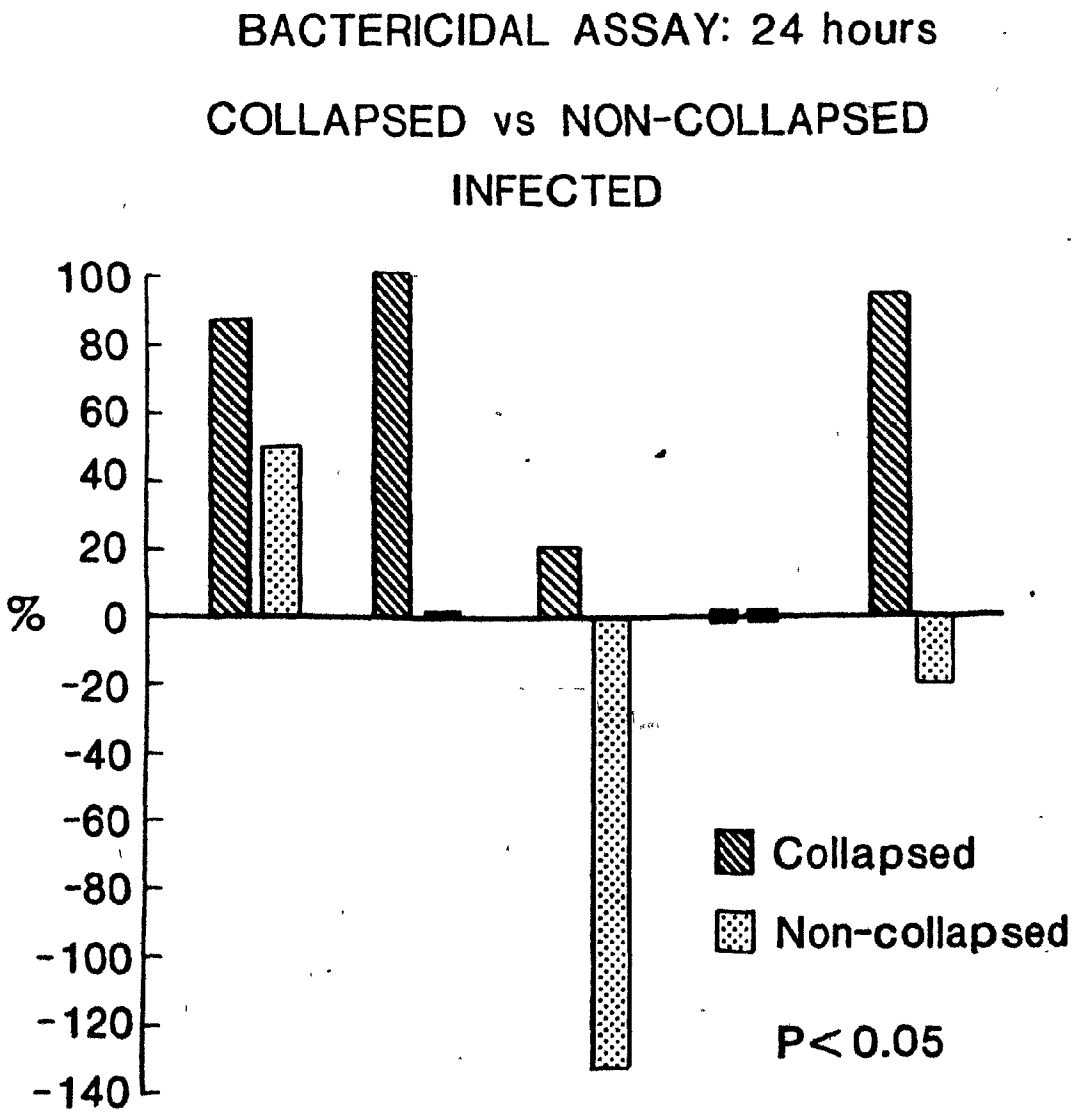

Figure XXX: Bactericidal (intracellular killing) capacity of AMs from infected collapsed lung versus control at 24 hours. The capacity is significantly increased in the collapsed lung specimens relative to the latter.

DISCUSSION

-Model Design:

Our results show that the distribution of aerosolized bacteria is even throughout all lobes. Trinkle, who first used this method, obtained the same even distribution results (146).

The techniques of performing a percutaneous lung biopsy and of cannulating the right lymphatic duct through the neck were used to cause the least disruption of respiratory mechanics. As we discussed, the act of ventilation itself effects the lymph flow and a splinted chest wall motion secondary to a thoracotomy would cause significant interference.

An original objective to investigate the importance of the mucociliary clearance had to be abandoned. By comparing bacterial clearance rates in the endobronchial collapse to the pneumothorax collapse, we had hoped to answer this question. However, the severe hemodynamic compromise which ensued from an adequate pneumothorax made the technique prohibitive. We certainly recognize the drawbacks in not examining this important clearance pathway. Although such a deficiency remains clinically relevant, as patients intubated for surgery or supported for respiratory failure, have lost their mucociliary clearance capability.

MICROBIOLOGY:

-Bacterial Clearance:

From our data it would appear that the collapsed lung, under the conditions of our experiment, has a decreased bacterial clearance

capacity. This impairment was evident from the first hour to the 24th and would imply that the dysfunction was immediately and "permenantly" induced.

Since this technique of measuring bacterial clearance has been in use, a large effort has been made to discover the most important mechanisms. Stossl (167) feels that the net rate of bacterial clearance from the lung is dependant on: rate of phagocytosis, bacterial killing, physical removal, and lastly bacterial multiplication. He as does Kavet (102) believes that the two most important factors are the first 2, and that clearance per se is very secondary.

We have found on the contrary that the bacterial clearance has decreased in the collapsed lung, in spite of the fact that phagocytosis and bactericidal killing were increased. Therefore, we looked at the concept of "exit pathways", available to the lung.

Also, our findings of decreased bacterial clearance are at variance with Trinkle and his group (146) who found that contusion alone, caused by external chest trauma, did not cause a depressed bacterial clearance. Using essentially the same infusion technique, they induced contusion by firing a 22-cal blank against a quarterplaced on the chest wall. Although there is a clear similarity to our study, two important distinctions must be made. First, in our study, bacteria were instilled as a single strain, to obviate any effects of competitive inhibition or facilitation. And second, our collapse of the lung parenchyma was considerably more extensive and prolonged. Thus possibly accounting for the decreased clearance effects with collapse alone, in our work.

BLOOD:

From our review work, intravascular exposure to bacteria, either systemically or at the lung level, probably provides a very important pathway to and from the lung RE system. On the other hand, bacteria administered into the alveoli in sufficient quantities may also utilize this pathway. The consequences of introducing bacteria into the blood stream at the relatively slow rate that must occur, because of the rapid blood flow, might indeed be very beneficial as a "dilution" effect.

- It is well known that intact proteins, albumin in particular, can be absorbed intact across the air tissue interface (Bensch, Staub, Vaughan, Lymphology). Indeed, Vaughan showed as we saw in our review that pulmonary blood absorbs 11 times the albumin than does pulmonary lymph from the alveoli. Figure XXXI is a possible explanation for this, demonstrating the proximity of the alveolus on the left to the pulmonary capillary endothelium as shown in this EM.

Our results were 2/9 blood cultures positive and therefore, no definite conclusion can be drawn. The site of sampling may be important to detect the low levels of bacterium as I pointed out above. A more appropriate site for sampling the blood as a pulmonary clearance mechanism would be the left heart. We elected not to pursue this, although future work in this area might very fruitful. It is doubtful that the external jugular blood was contaminated by efferent lymphatics, as the lymph specimens were not simultaneously positive.

Figure XXXI: Electronmicrograph of the alveolo-capillary junction; EP is a type I pneumocyte, and is separated from the pulmonary capillary endothelium by the basement membrane (BM). (From Stossl 167).

LYMPH:

Van Ord Alblas (13) theorized that 2 major exit pathways exist in the lung - airways and mucociliary, and lymphatics. The latter is processed by bronchus associated lymphoid tissue (BALT) as seen in figure XXXII and taken from one of the animals. From here, the bacteria within AMs may be removed into airways at a higher level, or by efferent lymphatics. As we have seen the lymph in the infected collapsed situation contained AMs, the possibility exists that bacteria so removed was viable or "escaped" to be recoverable by culture. We would have to conclude as in the case of the blood, that both may serve as significant exit pathways, although certainly in the case of the lymphatics, our data would not strongly support this.

MUCOCILIARY:

On balance, by a process of deduction, we feel that the mucociliary clearance is very important for bacterial clearance. Indeed, it might be the main reason for the depressed bacterial clearance. The same conclusion may, therefore, be valid particularly for atelectasis where terminal airways, as well as alveoli collapse.

Figure XXXII: Bronchus associated lymphoid tissue or BALT. Tissue was removed from a pig at the completion of a 24 hour experiment.

PULMONARY LYMPHATICS:

-Infected-Collapsed:

The significant increases in lymphatic flow rate as well as the lymph to plasma protein ratio in response to the infection collapse, characterize the transudation as high permeability edema. As we have seen, Brigham and Staub obtained the same results with intravenous E Coli. In Brigham's study, the lymph flow increased in this manner 5 times the baseline value (21). By aerosolized introduction, we obtained even greater increases of up to 10 times the baseline values.

It would appear that permeability response may reflect the opsonization needs of the bacteria - particularly those encapsulated, as in this case. In our work, we found that the inoculation of Strep Pneumoniae and E Coli both caused a rapid influx of polymorphonuclear cells as well as a stimulation of the macrophage. This increase which we noted, particularly in the lungs which were collapsed over the non-collapsed side, may again represent the influx of opsonins mainly in the form of complement. Why there is an increase in opsonic requirement on the collapsed side is unexplained. Goldstein (62) theorizes that there is a critical phagocyte to bacteria ratio, after which the release of soluble factors by the AM cause an influx of PMNs and complement factors, and a resultant increase in permeability. This may be one factor.

Hof (81,82) examined the opsonic requirements of Staph. Aureus and Strep Pneumoniae and found that alveolar macrophages engulfed greater than 90% of the Staph. Aureus which was pre-opsonized in 1% albumin that had been heated to destroy complement. Indeed, he showed that

the small amounts of protein normally found in the alveolar space would be suitable opsonins for alveolar macrophage for Staph. Aureus. However, in the case of Strep Pneumoniae, he found, as we did, that the alveolar macrophage was unable to effectively phagocytize the microbe without complement. We will refer to this in the section on phagocytosis.

There is increased cellular content of the efferent lymph, as we have seen, in the form of RBCs, neutrophils, and AMs, the numbers of which are not normally present. T-lymphocytes, on the other hand, are. This picture is again consistent with an increased permeability in the alveolo-capillary membrane with a prominent hemorrhagic component. As early as 1942, Drinker and Warren (149) described this hemorrhagic component to the efferent lymph in a similar situation.

ALVEOLAR MACROPHAGES:

-Morphology:

By light histology, the collapsed lung AM specimens showed increased vacuolizations. This finding corresponded to the EM work that identified increases in lysosomal inclusions and an increase in the pseudopodia. In essence, the AM from the infected collapsed lung is histologically more primed for endocytosis, in a type of overdrive as it were. Indeed, at 24 hours, this AM lacks a great deal of support organelles in the form of mitochondria and endoplasmic reticulum, as we saw on the EM.

Conspicuous by number in the infected collapsed specimen are the polymorphonuclear cells. Their presence there, in much greater number

than the control specimen, are a reflection of the opsonic needs that we discussed above. Indeed, many of the works we reviewed support this view that the extracellular Pneumococcus, with the specific capsular polysaccharide, required opsonization and phagocytosis by PMNs as well as AMs (72,81).

-Cell Count and Differential:

The rebound in numbers of AMs from 6 to 24 hours implies a replenishment source. Many workers, notably Golde and Alblas, have attempted to put a time as well as source to this. From our review, replacement from both circulating monocytes as well as local lung replication is possible within this time period, although too short for a bone marrow source.

The differential of the lavage specimen from the infected collapsed lungs showed a decrease in % relative to the other cells in the fluid. The total absolute number was greater than the control number. This is again consistent with the influx of neutrophils, and immunoglobulins, such as IgG to act as opsonins - all in response to instilled bacteria.

Of interest, is the fact that the AM from the non-infected collapsed did not increase in number over the control. One can conclude that the lack of bacterial stimulation is the reason. Also, the differential % of AMs did not vary consistently as was the case with lavage from the infected collapsed lung.

The effects of collapse alone and in particular, pretreatment with indomethacin while deserving of note in our results, any conclusions

drawn would be unwarranted. Of interest are the clinical implications for controlling fluid transudation through prostaglandin manipulation. Certainly, the area warrants further complete investigation.

Harmsen's study on pig AMs (74) revealed that the differentials for the normals or controls were 54% alveolar macrophage, 24% lymphocytes and 22% heterophilic cells. T-gondii-infected was 62%, 33% and 5% respectively and the Freund's Adjuvant stimulated greater than 85% alveolar macrophage production. These cell differentials are somewhat higher than our findings, where we found an average in our controls of 38.91% of macrophages and in our infected collapsed lung segment of 33.5% and non-collapsed 45.98%.

ALVEOLAR MACROPHAGE FUNCTION:

-Chemotaxis:

The relative depressed chemotaxis with the AM from the collapsed as opposed to the non-collapsed lung is not incompatible with the metabolic byproducts. See figure XXXIII. The intermediates of a superoxide anion in abundance, is known to be a chemotactic depressant factor. Unquestionably, the AM from the collapsed side is more active as measured by histology, and by function studies, consequently, its intermediates may be in relatively greater concentration than the other side.

Also, high levels of the chemotactic factor C5a cause a depression in AM chemotaxis over a period of exposure as we reviewed earlier.

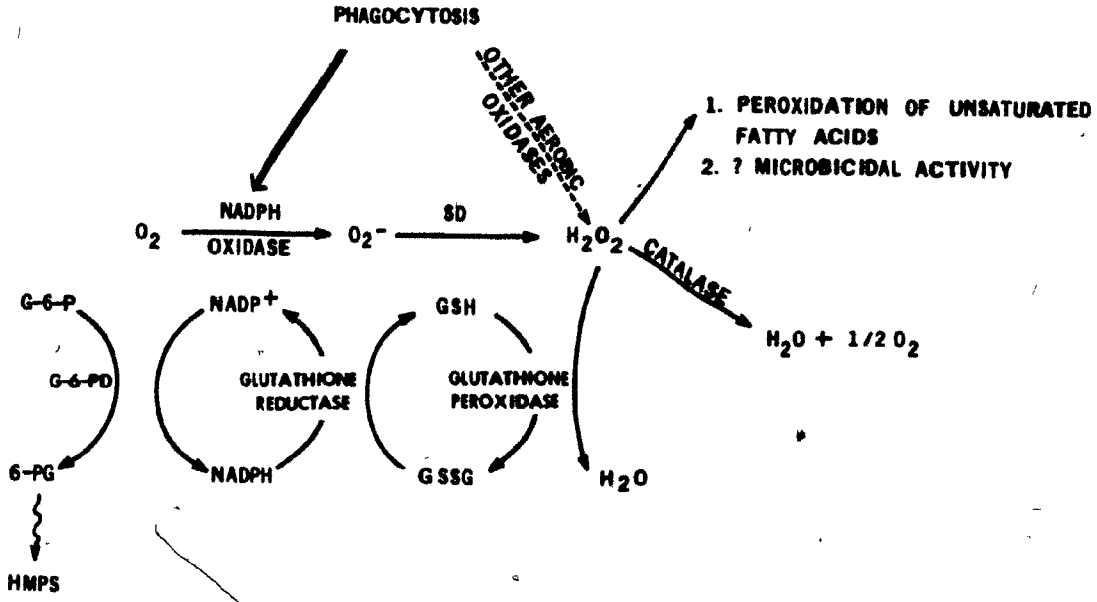

Figure XXXIII: Diagram of the metabolic energy pathways available to the AM (from Stossl).

Technical problems with testing includes 2 main areas - chemotactic factor choice and filter system. Both reflect the problems with utilizing previous data, much of which is drawn from the peritoneal or other species macrophage experience.

We experimented with tripeptides with which Dauber (35) reported very good results in guinea pig alveolar macrophages. He showed that they were more effective than bacterial chemotactic factor which was more effective than lymphocyte derived chemotactic factor which was more effective than endotoxic activated guinea pig serum. He used n-formyl methionyl alanine, n-formyl methionyl phenylalanine: both showed stimulation for migration of peritoneal macrophages and alveolar macrophage results were variable. However, the peptide n-formyl methionyl phenylalanine showed specific orientation to the direction of migration of macrophages in vitro which the author took as indicating as truly chemotactic.

We attempted to reproduce these findings with the exact same peptides and we used them at all reasonable dilution strengths, however, we were totally unable to demonstrate superiority of these chemotactic factors over endotoxin-activated serum. One explanation, of course, is species difference. Another one is that these authors did not make the distinction in their study between chemokinesis and chemotaxis, and that this could explain why they both had variable responses and had orientation that might well have been fortuitous. Other explanations are their techniques in collecting the macrophages from the peritoneum and from the lung,and the manner of handling which certainly select out

sub-populations that might be distinctly responsive to various factors.

Present literature on macrophage function and in vitro testing, has a basis in work on the peritoneal macrophage, most probably because it is so readily available, particularly with the mouse. Technically, they are certainly more easily retrieved. This presented some problems in interpreting and formulating the data for setting up our laboratory during the year to examine the alveolar macrophage. Dohlman's work (40) in which he examined the in vitro migration of both rabbit alveolar and peritoneal macrophages quantitated chemotaxis by agarose well assay. He compared them under the stimulation of rabbit serum, tryptic fragments of the C5 and peptide which we utilized and I referred to earlier as formyl methionyl phenylalanine leucine. Throughout, the peritoneal macrophage exhibited greater chemotaxis than the alveolar. As well, chemokinesis was much greater by factors of 2 to 300 than was the chemotaxis measurement, particularly in the alveolar macrophage.

In essence then, the heterogeneity of the monocytes, a concept that we explored in our review, has to be kept in mind when relating testing information between different monocyte subgroups.

Further technical changes are utilizing an agarose plate system which we are currently transferring to. This eliminates mechanical barriers that the pores may have to the somewhat larger (than peritoneal) AMs. Combining this gel filtration technique with the use of lung lining

material or the cell free material may improve our results to the significant levels. Schwartz (152) showed very good lung lining influence on AM chemotaxis.

A future test which certainly looks simpler and which we may endeavour to utilize is that comparable to our phagocytosis test, that is using Cr labeled radioassay. Gallin (52) demonstrates this as a time-saving technique with a great variability in range and capable of being easily standardized. He showed that the radiation from the chromium did not influence chemotaxis.

PHAGOCYTOSIS:

The unique capacity of the AM to function by both aerobic and anaerobic pathways (figure XXXIII) are probably the basis for its increased phagocytosis under relatively hypoxic conditions. As well, the increased phagocytosis in the AM from the infected collapsed might be a reflection of the increased opsonins available for phagocytosis. This is again compatible with the observed cellular and histologic changes.

Harmsen's pig AM work showed their phagocytosis levels were very compatible with ours. They found in the normals that they had a phagocytosis level of 21%, of their FCA of 20% and of their T-gondii of 19% and that only sheep red blood cells opsonized with IgG were capable of being endocytized or phagocytized. The addition of complement enhanced the number of the phagocytized particles by approximately 50%. Likewise, he demonstrated that there are FC and complement receptors on the alveolar macrophage and that 90%

of the porcine alveolar macrophages have IgG receptors. This is similar to the guinea pig, the rabbit, and the human. Certainly, the phagocyte-opsonin interaction could account for the relative increase.

One important feature that distinguishes macrophages from other cells, and which sometimes becomes an impediment to their study, is adherence. The macrophage strongly adheres to most surfaces, particularly glass, to form so called mono-layers, this, in distinction to lymphocytes. This feature helps to obtain the cells from the lavage aspirate, on the one hand, but on the other, may exclude adhered cells from proper inclusion in certain in vitro tests. This is particularly true of phagocytosis, and we examined the inherent difficulties and inaccuracies with previous testing protocols in our research by the tube-well interchange. The increase in phagocytosis % with the tube, we feel, reflects the effects of adherence, and gives a more accurate result, than utilizing the well system.

BACTERICIDAL:

A "surprise" finding for us was the significantly increased bactericidal capacity in the AM from the infected collapsed versus the control. This particularly, as the bacterial clearance was depressed in this group.

From the morphologic, functional features of these very stimulated AMs, it would reasonably follow that this function because of the increased hydrogen peroxide formation (figure XXXIII) would be

increased. However, this is counter to the literature as we have pointed out, that a defect in pulmonary intracellular killing is more important than clearance (Stossl) pathways. This finding again mitigates for the importance of these pathways, particularly mucociliary. The obstructive component of atelectasis might, therefore, account for the decreased bacterial clearance more than an AM effect as they are apparently so metabolically adaptable.

We also noted the technical point that our in vivo bacterial challenge could be effectively used in this test, in place of the usual in vitro test bacteria. Results from both were compatible, and perhaps greater experimental utilization will be made of this test in instances of localized pulmonary infections.

REFERENCES

1. Acton, J.D., Myrvik, Q.N.: Production of Interferon by Alveolar Macrophages. J. of Bacteriol. 91: 2300-2304, 1966.

2. Addonizio, Jr., V.P., Harken, A.H.: The Surgical Implications of Nonrespiratory Lung Function. J. of Surg. Res. 28: 86-95, 1980.

3. Alitalo, K., Hovi, T., Vaheri, A.: Fibronectin is Produced by Human Macrophages. J. Exp. Med. 151: 602-613, 1980.

4. Arenson, Jr., E.B., Epstein, M.B., Seeger, R.C.: Volumetric and Functional Heterogeneity of Human Monocytes. J. Clin. Invest. 65: 613-618, 1980.

5. Aronin, P.A., Mahaley, Jr., M.S., Rudnick, S.A. et al: Prediction of BCNU Pulmonary Toxicity in Patients with Malignant Gliomas. New England J. of Med. 303(4): 183, 1980.

6. Austriam, R.: Random Gleanings from a Life with the Pneumococcus. J. Infect. Dis. 131:474, 1975.

7. Baker, C.J., Kasper, D.L., Edwards, M.S. et al: Influence of Preimmunization Antibody Levels on the Specificity of the Immune Response to Related Polysaccharide Antigens. New England J. of Med. 303(4):173, 1980.

8. Barker, M., Deen, D.F., Baker, D.G.: BCNU and X-Ray Therapy of Intracerebral Rat Tumors. Int. J. Radiat. Oncol. Biol. Phys. 5:1581-3, 1979.

9. Baum, O.S., Amberson, Jr., J.B.: Nontubercular Pulmonary Infections Complication Pulmonary Tuberculosis. Am. Rev. Tuberculosis, 45:243-279, 1942.

10. Bensch, K.G., Dominguez, E., Liebow, A.A.: Absorption of Intact Protein Molecules across the Pulmonary Air-Tissue Barrier. Science, 157:1204, 1967.

11. Berman, I.R., Iliescu, H., Ranson, J.H.C. et al: Pulmonary Capillary Permeability - A Transfusion Lesion. J. of Trauma, 16:471, 1976.

12. Bice, D.E., Harris, D.L., Hill, J.O. et al: Local and Systemic Immunity following Localized Deposition of Antigen in the Lung. Chest, 75:247, 1979.

13. Blussé Van Oud Alblas, A., Van Furth, R.: Origin, Kinetics, and Characteristics of Pulmonary Macrophages in the Normal Steady State. J. Exp. Med. 149:1504, 1979.

14. Boorman, G.A., Schwartz, L.W., Wilson, F.D.: Formation of Macrophage Colonies In Vitro by Free Lung Cells Obtained from Rats. J. of the Reticuloendothelial Soc. 26:855, 1979.

15. Boorman, G.A., Schwartz, L.W., Wilson, F.D.: In Vitro Macrophage Colony Formation by Free Lung Cells During Pulmonary Injury. J. of the Reticuloendothelial Soc. 26:867, 1979.

16. Bowles, A.L., Dauber, J.H., Daniele, R.P.: The Effect of Hyperoxia on Migration of Alveolar Macrophages In Vitro. Am. Rev. of Resp. Dis. 120:541, 1979.

17. Bradley, P.A., Bourne, F.J., Brown, P.J.: The respiratory Tract Immune System in the Pig. I. Distribution of Immunoglobulin-Containing Cells in the Respiratory Tract Mucosa. Vet. Pathol. 13:81-89, 1976.

18. Bradley, P.A., Bourne, F.J., Brown, P.J.: The Respiratory Tract Immune System in the Pig. II. Associated Lymphoid Tissues. Vet. Pathol. 13:90-97, 1976.

19. Bredenberg, C.E., Kazui, T., Webb, W.R.: Experimental Pulmonary Edema: The Effect of Positive End-Expiratory Pressure on Lung Water. Annals of Thoracic Surg. 26:62, 1978.

20. Bressack, M.A., McMillan, D.D., Bland, R.D.: Pulmonary Oxygen Toxicity: Increased Microvascular Permeability to Protein in Unanesthetized Lambs. Lymphology, 12:133-139, 1979.

21. Brigham, K.L.: Pulmonary Edema: Cardiac and Non-Cardiac. Am. J. of Surg. 138:361, 1979.

22. Brigham, K.L., Harris, Th.R., Bowers, R.E. et al: Lung Vascular Permeability: Inferences from Measurements of Plasma to Lung Lymph Protein Transport. Lymphology, 12:177-190, 1979.

23. Cameron, D.J., Churchill, W.H.: Cytotoxicity of Human Macrophages for Tumor Cells: Enhancement by Bacterial Lipopolysaccharides (LPS), J. of Immunology, 124:708, 1980.

24. Carlson, R.W., Schaeffer, Jr., R.C., Michaels, S.G. et al: Pulmonary Edema Fluid - Spectrum of Features in 37 Patients. Circ. 60:1161, 1979.

25. Chang, Y., Yao, C.: Investigation of the Human Macrophage I. Collection and in vitro Cultivation. Eur. J. Immunol. 9:517-520, 1979.

26. Chang, Y., Yao, C.: Investigation of the Human Macrophage II. The in vitro Cytotoxicity of Macrophages. Eur. J. Immunol. 9:521-525, 1979.

27. Christou, N.V., Meakins, J.L.: Delayed Hypersensitivity in Surgical Patients: A Mechanism for Anergy. Surg. 86(1):78-85, 1979.

28. Collins, J.J., Snyderman, R.: Absence of Macrophage Involvement in the Passive Serum Therapy of Friend Leukemia Virus-induced Disease. Cancer Res. 40:557-562, 1980.

29. Collins, J.C., Harris, T.R., McKeen, C.R. et al: Increased Lung Lymph Transport without Heart Failure after Coronary Ligation in Sheep. J. Appl. Physiol. 47(4): 792, 1979.

30. Comroe, J.H., Forster, R.E., Dubois, A.B.: The Lung - Clinical Physiology and Pulmonary Function Tests, p. 61-65. Yearbook Med. Pub. Inc. Chicago.

31. Connell, R.S., Swank, R.L., Webb, M.C.: The Development of Pulmonary Ultrastructural Lesions During Hemorrhagic Shock. J. of Trauma, 15:116, 1975.

32. Crutchley, D.J., Boyd, J.A., Eling, T.E.: Enhanced Thromboxane B_2 Release from Challenged Guinea Pig Lung after Oxygen Exposure. Am. Rev. of Resp. Dis. 121:695, 1980.

33. Da Luz, P.L., Shubin, H., Weil, M.H. et al: Pulmonary Edema Related to Changes in Colloid Osmotic and Pulmonary Artery Wedge Pressure in Patients after Acute Myocardial Infarction. Circ. 51:350, 1975.

34. Dantzker, D.R., Brook, C.J., Dehart, P. et al: Ventilation-Perfusion Distributions in the Adult Respiratory Distress Syndrome. Am. Rev. of Resp. Dis. 120:1039, 1979.

35. Dauber, J.H., Daniele, R.P.: Chemotactic Activity of Guinea Pig Alveolar Macrophages. Am. Rev. of Resp. Dis. 117:673, 1978.

36. Davies, P., Sornberger, G.C., Huber, G.L.: A Method for the Collection of Alveolar Macrophages for Microscopy. Am. Rev. of Resp. Dis. 116:1113, 1977.

37. Demling, R.H., Manohar, M., Will, J.A.: Relationship between the Measured and Calculated Colloid Osmotic Pressure of Plasma and Lung Lymph in the Sheep. Lymphology, 13:18-23, 1980.

38. Demling, R.H., Mamohar, M., Will, J.A. et al: The Effect of Plasma Oncotic Pressure on the Pulmonary Microcirculation after Hemorrhagic Shock. Surg. 86:323, 1979.

39. Diamantstein, T., Oppenheim, J.J., Unanue, E.R. et al: Nonspecific "Lymphocyte Activating" Factors Produced by Macrophages. Clin. Immunol. and Immunopathol. 14: 264-267, 1979.

40. Dohlman, J.G., Goetzl, E.J.: Unique Determinants of Alveolar Macrophage Spontaneous and Chemokinetically Stimulated Migration. Cell. Immunology, 39:36, 1978.

41. Drake, R.E., Smith, J.H., Gabel, J.C.: Estimation of the Filtration Coefficient in Intact Dog Lungs. Am. J. Physiol. 238:H430, 1980.

42. Empey, D.W.: Diseases of the Respiratory System - Introduction: structure and function of the lungs. Br. Med. J. 1:631-633, 1978.

43. Fearon, D.T., Austen, K.F.: The Alternative Pathway of Complement System for Host Resistance. New Eng. J. of Med. 303(5) 259-62, 1980.

44. Finley, T.N.: Pulmonary Surfactant. Am. Rev. of Resp. Dis. 89:372-78, 1964.

45. Fishman, A.P.: Pulmonary Edema - The Water-Exchanging Function of the Lung. Circ. XLVI, 390, 1972.

46. Fishman, A.P., Pietra, G.G.: Handling of Bioactive Materials by the Lung. New Eng. J. of Med. 291:884, 1974.

47. Fonkalsrud, E.W.: Surgical Management of Congenital Lymphedema in Infants and Children. Arch. Surg. 114: 1133, 1979.

48. Fountain, S.W., Martin, B.A., Musclow, C.E. et al: Pulmonary Leukostasis and Its Relationship to Pulmonary Dysfunction in Sheep and Rabbits. Circ. Res. 46:175, 1980.

49. Fujiwara, T., Chida, S., Watabe, Y. et al: Artificial Surfactant Therapy in Hyaline-Membrane Disease. Lancet, 8159, Jan. 1980.

50. Fulton, R.L., Jones, C.E.: The Cause of Post-Traumatic Pulmonary Insufficiency in Man. Surg., Gyn. & Obs., 140:179, 1975.

51. Gadek, J.E., Hunninghake, G.W., Zimmerman, R.L. et al: Regulation of the Release of Alveolar Macrophage-Derived Neutrophil Chemotactic Factor. Am. Rev. of Resp. Dis. 121:723, 1980.

52. Gallin, J.I., Clark, R.A., Kimball, H.R.: Granulocyte Chemotaxis: An Improved in vitro Assay empolying 51-Cr-Labeled Granulocytes. J. of Immunology, 110:233, 1973.

53. Garnett, E.S., Webber, C.E., Coates, G, et al: Lung Density: clinical method for quantitation of pulmonary congestion and edema. CMA J. 116: 153, 1977.

54. Gee, M.H., Donovan, K.A.: The Flow and Composition of Pulmonary and Systemic Lymph in Dogs with Edema. Lymphology, 12:125-132, 1979.

55. Gee, M.H., Staub, N.C.: Role of bulk fluid flow in protein permeability of the dog lung alveolar membrane. J. Applied Physiol. 42(2) p144-149, 1977.

56. Gelb, A.F., Klein, E.: Hemodynamic and Alveolar Protein Studies in Noncardiac Pulmonary Edema. Am. Rev. of Resp. Dis. 114:831, 1976.

57. Gerdin, B., Saldeen, T.: Deposition and Clearance of Fibrin in the Rat Lung Following Acute Haemorrhage. Circulatory Shock, 6:357, 1979.

58. Glauser, F.L., Millen, J.E., Falls, R.: Increased Alveolar Epithelial Permeability with Acid Aspiration: The Effects of High-Dose Steroids. Am. Rev. of Resp. Dis. 120:1119, 1979.

59. Glauser, F.L., Millen, J.E., Falls, R.: Effects of Acid Aspiration on Pulmonary Alveolar Epithelial Membrane Permeability. Chest, 76:201, 1979.

60. Golde, D.W.: Alveolar Proteinosis and the Overfed Macrophage. Chest, 76:119, 1979.

61. Golde, D.W.: Kinetics and Function of the Human Alveolar Macrophage. J. of Reticuloendothelial Soc. 22:223, 1977.

62. Goldstein, E., Bartlema, H.C.: Role of the Alveolar Macrophage in Pulmonary Bacterial Defense. Bull. europ. Physiopath. resp. 13:57-67, 1977.

63. Gorin, A.B., Stewart, P.A.: Differential permeability of endothelial and epithelial barriers to albumin flux. J. Appl. Physiol. 47 :1315, 1979.

64. Gorin, A.B., Weidner, W.J., Demling, R.H. et al: Noninvasive measurement of pulmonary transvascular protein flux in sheep. J. Appl. Physiol. 45 :225, 1978.

65. Granger, H.J.: Role of the Interstitial Matrix and Lymphatic Pump in Regulation of Transcapillary Fluid Balance. Microvascular Res. 18:209-216, 1979.

66. Grant, M.M., Sorokin, S.P., Brain, J.D.: Lysosomal Enzyme Activities in Pulmonary Macrophages from Rabbits Breathing Iron Oxide. Am. Rev. of Resp. Dis. 120:1003, 1979.

67. Green, G.M., Kass, E.H.: The Role of the Alveolar Macrophage in the Clearance of Bacteria from Lung. J. Exp. Med. 119:167, 1964.

68. Grogan, J.B.: Transient Defect in the Bactericidal Capacity of Rabbit Alveolar Macrophages Following Sublethal Shock. J. of Trauma, 19:52, 1979.

69. Grogan, J.B., Lockard, V.: Acute Alterations in the Bactericidal Capacity of Rabbit Alveolar Macrophages Following Stress. J. of Trauma, 13:877, 1973.

70. Gudewicz, P.W., Saba, T.M., Coulston, F.: Phagocytic and Bactericidal Activities of Pulmonary Macrophages following Sublethal Traumatic Shock. Proc. of Soc. for Exp. Biol. and Med. 153:262-267, 1976.

71. Ham, A.W.: Histology, 6th edition, J.B. Lippincott, Philadelphia, 1969.

72. Hand, W.L., Cantey, J.R., Hughes, C.G.: Antibacterial Mechanisms of the Lower Respiratory Tract - I. Immunoglobulin Synthesis and Secretion. J. of Clin. Invest. 53:354, 1974.

73. Harfor, C.G., Leidler, V., Hara. M.: Effect of the Lesion due to the Influenza Virus of the Resistance of Mice to Pneumococcus. J. Exp. Med. 89:53-67, 1949.

74. Harmsen, A.G., Jeska, E.L.: Surface Receptors on Porcine Alveolar Macrophages and Their Role in Phagocytosis. J. of Reticuloendothelial Soc. 27:631, 1980.

75. Harris, J.O.: Pulmonary Alveolar Proteinosis - Abnormal in vitro Function of Alveolar Macrophages. Chest, 76: 156, 1979.

76. Harrison, Jr., L.H., Hinshaw, L.B., Coalson, J.J. et al: Effects of E Coli septic shock on Pulmonary Hemodynamics and Capillary Permeability. J. of Thoracic and Cardiovasc. Surg. 61:795, 1971.

77. Haslam, P.L., Turton, C.W.G., Heard, B. et al: Bronchoalveolar lavage in pulmonary fibrosis: comparison of cells obtained with lung biopsy and clinical features. Thorax, 35:9-18, 1980.

78. Hewson, J.R., Holland, A.J.C., McArdle, A.H.: Transalveolar Metabolic Protection to the Lung. Arch. Surg. 111:812, 1976.

79. Hinman, L.M., Stevens, C.A., Matthay, R.A. et al: Elastase and Lysozyme Activities in Human Alveolar Macrophages. Am. Rev. of Resp. Dis. 121:263, 1980.

80. Hocking, W.G., Golde, D.W.: The Pulmonary-Alveolar Macrophage (First of Two Parts). New Eng. J. of Med. 301:580, 1979.

81. Hof, D.G., Hoidal, J.R., Repine, J.E.: Phagocytosis by Human Alveolar Macrophages (AM) and Neutrophils (PMN). Chest, 76:371, 1979.

82. Hof, D.G., Repine, J.E., Peterson, P.K. et al: Phagocytosis by Human Alveolar Macrophages and Neutrophils: Qualitative Differences in the Opsonic Requirements for Uptake of Staphylocossus Aureus and Streptococcus Pneumoniae in vitro. Am. Rev. of Resp. Dis. 121:65, 1980.

83. Hoidal, J.R., White, J.G., Repine, J.E.: Impairment of Human Alveolar Macrophage Oxygen Consumption, and Superoxide Anion Production by Local Anesthetics Used in Bronchoscopy. Chest, 75:244, 1979.

84. Holt, P.G.: Inhibitory Activity of Unstimulated Alveolar Macrophages on T-Lymphocyte Blastogenic Response. Am. Rev. of Resp. Dis. 118:791, 1978.

85. Holt, P.G.: Alveolar Macrophages. I. A Simple Technique for the Preparation of High Numbers of Viable Alveolar Macrophages from Small Laboratory Animals. J. of Immunological Methods, 27:189, 1979.

86. Hopewell, P.C.: Failure of Positive End-Expiratory Pressure to Decrease Lung Water Content in Alloxan-Induced Pulmonary Edema. Am. Rev. of Resp. Dis. 120:813, 1979.

87. Horwitz, M.A., Silverstein, S.C.: Influence of the Escherichia Coli Capsule on Complement Fixation and on Phagocytosis and Killing by Human Phagocytes. J. Clin. Invest. 65:82, 1980.

88. Howard, R.J.: Host Defense Against Infection - Part I. Current Problems in Surgery, May, 1980. Vol. XVII (5).

89. Hsueh, W., Kuhn, C.S.: Prostaglandin Secretion in Rabbit Alveolar Macrophages and Its Relationship to Phagocytosis. Chest, 75:249, 1979.

90. Huber, G.L., Pochay, V.E., Pereira, W. et al: Marijuana, Tetrahydrocannabinol, and Pulmonary Antibacterial Defenses. Chest, 77:403, 1980.

91. Hunninghake, G.W., Gadek, J.E., Young, Jr., R.C. et al: Maintenance of Granuloma Formation in Pulmonary Sarcoidosis by T-Lymphocytes within the Lung. New Eng. J. of Med. 302:594, 1980.

92. Hunninghake, G.W., Gallin, J.I., Fauci, A.S.: Immunologic Reactivity of the Lung - The in Vivo and in Vitro Generation of a Neutrophil Chemotactic Factor by Alveolar Macrophages. Am. Rev. of Resp. Dis. 117:15, 1978.

93. Hunter, R.L., Enker, W.E., Cote, R.J. et al: Macrophage-Related Skin Test Reactivity in Normal and Tumor-Bearing Hosts. J. of Reticuloendothelial Soc. 27:23, 1980.

94. Jacob, H.S., Craddock, P.R., Hammerschmidt, D. E. et al: Complement-Induced Granulocyte Aggregation. New Eng. J. of Med. 302:789, 1980.

95. Jakab, G.J., Greer, G.M.: Pulmonary Defense Mechanisms in Consolidated and Nonconsolidated Lungs Infected with Sendai Virus. J. Infect. Dis. 129:263-269, 1974.

96. Jakab, G.J., Warr, G.A., Sannes, P.L.: Alveolar Macrophage Ingestion and Phagosome-Lysosome Fusion Defect Associated with Virus Pneumonia. Infect. and Immunity, 27:960, 1980.

97. Jawetz, E., Melnick, J., Adelberg, E.: Review of Medical Microbiology, 13th Edition, Lange Medical Publication, Los Altos, California, 1978.

98. Johnson, J.D., Hand, W.L., Francis, J.B. et al: Antibiotic uptake by alveolar macrophages. J. Lab. Clin. Med. 95:429, 1980.

99. Jones, J.G., Lawler, P., Crawley, J.C.W. et al: Increased Alveolar Epithelial Permeability in Cigarette Smokers. Lancet, 318:66, Jan. 1980.

100. Kaplan, J.E., Saba, T.M.: Humoral deficiency and Reticuloendothelial Depression after Traumatic Shock. Am. J. of Physiol. 230:7, 1976.

101. Kaufman, H.S., Leeds, S.E., Uhley, H.N. et al: T-Lymphocytes in Venous Blood and Lymph from the Thoracic Duct and Right Lymphatic Duct of Normal Dogs. Lymphology, 13:40-43, 1980.

102. Kavet, R.I., Brain, J.D.: Phagocytosis: quantification of rates and intercellular heterogeneity. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 42:432, 1977.

103. Kavet, R.I., Brain, J.D.: Methods to Quantify Endocytosis: A Review. J. of Reticuloendothelial Soc. 27:201, 1980.

104. Laforce, F.M., Mullane, J.F., Boehme, R.F. et al: The Effect of Pulmonary Edema on Antibacterial Defenses of the Lung. J. Lab. Clin. Med. 82:634, 1973.

105. Landa, J. F., Kwoka, M.A., Chapman, G.A. et al: Effects of Suctioning on Mucociliary Transport. Chest, 77:202, 1980.

106. Lawrence, E.C., Blaese, R.M., Martin, R.R. et al: Immunoglobulin Secreting Cells in Normal Human Bronchial Lavage Fluids. J. of Clin. Invest. 62: 832, 1978.

107. Lewis, F.R., Elings, V.B., Sturm, J.A.: Bedside Measurement of Lung Water. J. of Surg. Res. 27:250, 1979.

108. Low, R.B.: Macromolecule Synthesis by Alveolar Macrophages: Response to a Phagocytic Load. J. of Reticuloendothelial Soc. 22:99, 1977.

109. Mackowiak, P.A.: Microbial Synergism in Human Infections (First of two parts). New Eng. J. of Med. 298:21, 1978.

110. Malik, A.B., van der Zee, H., Lee, B.C.: Pulmonary Transvascular Fluid Dynamics in Sheep During Hemorrhage. Lymphology, 12:149, 1979.

111. Manawadu, B.R., LaForce, F.M.: Impairment of Pulmonary Antibacterial Defense Mechanisms by Halothane Anesthesia. Chest, 75:242, 1979.

112. Markham, R.J.F., Wilkie, B.N.: Interaction Between Pasteurella Haemolytica and Bovine Alveolar Macrophages: Cytotoxic Effect on Macrophages and Impaired Phagocytosis. Am. J. Vet. Res. 41:18, 1980.

113. Mathé, A.A., Hedqvist, P., Strandberg, K. et al: Aspects of Prostaglandin Function in the Lung. (First of two parts). New Eng. J. of Med. 296:850, 1977.

114. McNamee, J.E., Staub, N.C.: Pore Models of Sheep Lung Microvascular Barrier Using New Data on Protein Tracers. Microvascular Res. 18:229, 1979.

115. Merrill, W.W., Naegel, G.P., Matthay, R.A. et al: Alveolar Macrophage-derived Chemotactic Factor - Kinetics of in vitor production and partial characterization. J. Clin. Invest. 65:268, 1980.

116. Meuret, G., Schildknecht, O., Joder, P. et al: Proliferation Activity and Bacteriostatic Potential of Human Blood Monocytes, Macrophages in Pleural Effusions, Ascites, and of Alveolar Macrophages. Blut, 40:17, 1980.

117. Meyer, E.C., Dominguez, E.A.M., Bensch, K.G.: Pulmonary Lymphatic and Blood Absorption of Albumin from Alveoli. Lab. Invest. 20:1, 1969.

118. Miller, R.D., Kueppers, F., Offord, K.P.: Serum concentrations of C3 and C4 of the Complement System in Patients with Chronic Obstructive Pulmonary Disease. J. Lab. Clin. Med. 95:266, 1980..

119. Modry, D.L., Chiu, C-J., Hinchey, E.J.: The roles of Ventilation and perfusion in lung metabolism. J. of Thoracic and Cardiovasc. Surg. 74:275, 1977.

120. Modry, D.L., Chiu, R. C-J.: Pulmonary Reperfusion Syndrome. An. of Thoracic Surg. 27:206, 1979.

121. Montazeri, G., Ghiorazzi, N., Fu, S.M. et al: Regulatory Role of Circulating Monocytes in the Differentiative and Proliferative Responses of Human B-Lymphocytes. Clin. Immunology and Immunopathol. 16:1-10, 1980.

122. Moore, V.L., Myrvik, Q.N.: The Role of Normal Alveolar Macrophages in Cell-Mediated Immunity. J. of Reticuloendothelial Soc. 21:131, 1977.

123. Muir, A.L., Flenley, D.C., Kirby, B.J. et al: Cardiorespiratory effects of rapid saline infusion in normal man. J. of Appl. Physiol. 38:786, 1975.

124. Murphey, S.A., Root, R.K., Schreiber, A.D.: The Role of Antibody and Complement in Phagocytosis by Rabbit Alveolar Macrophages. J. of Infect. Dis. 140:896, 1979.

125. Murphy, S., Van Epps, D.E.: Neutrophil Chemiluminescence Defect in Pediatric Patients with Recurrent Pneumonias. Chest, 75:254, 1979.

126. Nadir, R.W.B., Gleisner, J., Hildebrandt, J.: Chronic Lung Lymph Fistula in the Goat. J. Appl. Physiol. 48:399, 1980.

127. Nathan, R.A., Kinsman, R.A., Spector, S.L. et al: J. Allergy Clin. Immunology, 64:491, 1979.

128. Newhouse, M., Sanchis, J., Bienenstock, J.: Lung Defense Mechanisms (First of two parts). New Eng. J. of Med. 295:990, 1976.

129. Nicolaysen, G., Hauge, A.: Determinants of transvascular fluid shifts in zone I lungs. J. Appl. Physiol. 48:256, 1980.

130. Nieman, G.F., Clark, Jr., W.R., Wax, S.D. et al: The Effect of Smoke Inhalation on Pulmonary Surfactant. Annals of Surg. 191:171, 1980.

131. Noble, W.H., Kay, J.C., Ordrzalek, J.: Mechanics in Hypervolemic Pulmonary Edema. J. Appl. Physiol. 38:681, 1975.

132. Ohkuda, K., Nakahara, K., Weidner, W.J. et al: Lung Fluid Exchange after Uneven Pulmonary Artery Obstruction in Sheep. Circ. Res. 43(2):152-61, 1978.

133. Okada, Y., Ito, M., Nagaishi, Ch.: Anatomical study of the Pulmonary Lymphatics. Lymphology, 12:118, 1979.

134. Overland, E.S., Nolan, A.J., Hopewell, P.C.: Alteration of Pulmonary Function in Intravenous Drug Abusers - Prevalence, Severity, and Characterization of Gas Exchange Abnormalities. Am. J. of Med. 68:231, 1980.

135. Pareti, F.I., Mannucci, P.M., D'Angelo, A.: Congenital Deficiency of Thromboxane and Prostacyclin. Lancet, 318:898, April, 1980.

136. Phan, S.H., Thrall, R.S., Ward, P.A.: Bleomycin-Induced Pulmonary Fibrosis in Rats: Biochemical Demonstration of Increased Rate of Collagen Synthesis. Am. Rev. of Resp. Dis. 121:501, 1980.

137. Polk, Jr., H.C.: Further Clarification of the Pathogenesis of Pulmonary Failure in Humans. Surg., Gyn. & Obs. 150:727, 1980.

138. Powanda, M.C., Bostian, K.A., Dinterman, R.E. et al: Phagocytosis and the Metabolic Sequelae of Infection. J. of Reticuloendothelial Soc. 27:67, 1980.

139. Prichard, J.S., Lee, G. de J.: Measurement of Water Distribution and Transcapillary Solute Flux in Dog Lung by External Radioactivity Counting. Clin. Sci. Mol. Med. 57(2):145-54, 1979.

140. Puchelle, E., Zahm, J-M., Bertrand, A.: Influence of Age on Bronchial Mucociliary Transport. Scand. J. Resp. Dis. 60:307, 1979.

141. Repine, J.E., Davies, S.F., Hoidal, J.R. et al: Effect of C2-Deficiency on the Bactericidal Activity and Chemiluminescence Responses of Human Neutrophils in vitro. Chest, 75:252, 1979.

142. Reynolds, H.Y.: Lung Host Defenses: A Status Report. Chest, 75:239, 1979.

143. Reynolds, H.Y., Thompson, R.E.: Pulmonary Host Defenses - I. Analysis of Protein and Lipids in Bronchial Secretions and Antibody Responses after Vaccination with Pseudomonas Aeruginosa. J. of Immunlogy, 111:358, 1973.

144. Reynolds, H.Y., Fulmer, J.D., Kazmierowski, J.A. et al: Analysis of Cellular and Protein Content of Broncho-Alveolar Lavage Fluid from Patients with Idiopathic Pulmonary Fibrosis and Chronic Hypersensitivity Pneumonitis. J. of Clin. Invest. 59:165, 1977.

145. Rice, C.L., Doroghazi, P.M., Kohler, J.P. et al: The Effect of Sepsis and Reduced Colloid Osmotic Pressure on Pulmonary Edema. J. of Surg. Res. 27:347, 1979.

146. Richardson, J.D., Woods, D., Johanson, Jr., W.G. et al: Lung bacterial clearance following pulmonary contusion. Surg. 86:730, 1979.

147. Robin, E.D., Cross, C.E., Zellis, R.: Pulmonary Edema (First of two parts), New Eng. J. of Med. 288:239, 1973.

148. Roubin, R., Zolla-Pazner, S.: Markers of macrophage heterogeneity - I. Studies of macrophages from various organs of normal mice. Eur. J. Immunol. 9:972, 1979.

149. Rusznyak, I., Foldi, M., Szabo, G.: Lymphatics and Lymph Circulation - Physiology and Pathology. Pergamon Press, New York, 1967.

150. Sbarra, A.J., Karnovsky, M.L.: The Biochemical Basis of Phagocytosis. J. Biol. Chem. 234:1355-62, 1959.

151. Schuyler, M.R., Gaumer, H.R., Stankus, R.P. et al: Bronchoalveolar Lavage in Silicosis. Lung, 157:95, 1980.

152. Schwartz, L.W., Christman, C.A.: Alveolar Macrophage Migration - Influence of lung lining material and acute lung insult. Am. Rev. of Resp. Dis. 120:429, 1979.

153. Shenker, B.J., Willoughby, W.F., Hollingdale, M.R. et al: In vivo Responses to Inhaled Proteins - III. Inhibition of Experimental Immune Complex Pneumonitis after Suppression of Peripheral Blood Lymphocytes. J. of Immunology, 124:1763, 1980.

154. Sibbald, W., Myers, M.L., Moffat, J. et al: Demonstrations of the Clinical Significance of Intravascular Starling Forces in Human Cardiac and Non-Cardiac Edema. Chest, 76:367, 1979.

155. Simon, L.M., Robin, E.D., Phillips, J.R.: Enzymatic Basis for Bioenergetic Differences of Alveolar versus Peritoneal Macrophages and Enzyme Regulation by Molecular 02. J. Clin. Invest. 59:443-48, 1977.

156. Simon, D.S., Murray, J.F., Staub, N.C.: Measurement of Pulmonary Edema in Intact Dogs by Transthoracic X-ray Attenuation. J. Appl. Physiol. 47:1228, 1979.

157. Simon, L.M., Liu, J., Theodore, J. et al: Effect of Hyperoxia, Hypoxia, and Maturation on Superoxide Dismutase Activity in Isolated Alveolar Macrophages. Am. Rev. of Resp. Dis. 115:279, 1977.

158. Simionescu, N.: Enzymatic Tracers in the Study of Vascular Permeability. J. of Histochemistry and Cytochemistry, 27:1120, 1979.

159. Slutsky, A.S., Scharf, S.M., Brown, R. et al: The Effect of Oleic Acid-Induced Pulmonary Edema on Pulmonary and Chest Wall Mechanics in Dogs. Am. Rev. of Resp. Dis. 121:91, 1980.

160. Smith, F.B., Kikkawa, Y., Diglio, C.A. et al: The Type II Epithelial Cells of the Lung - VI. Incorporation of 3H-Choline and 3H-Palmitate into Lipids of Cultured Type II Cells. Lab. Invest. 42:296, 1980.

161. Soter, N.A., Wasserman, S.I., Austen, K.F. et al: Release of Mast-Cell Mediators and Alterations in Lung Function in Patients with Cholinergic Urticaria. New Eng. J. of Med. 302:604, 1980.

162. Staub, N.C.: Cecil K. Drinker: Pioneer Lymphologist. Lymphology, 12:115, 1979.

163. Staub, N.C.: Pulmonary Edema due to Increased Microvascular Permeability to Fluid and Protein. Circ. Res. 43:143, 1978.

164. Staub, N.C.: Pulmonary Edema - Hypoxia and Over-Perfusion, New Eng. J. of Med. 302:1085, 1980.

165. Staub, N.C., Gee, M., Vreim, C.: General Discussion II - Mechanism of alveolar flooding in acute pulmonary edema. Lung Liquids, (Ciba Foundation Symposium 38), Elsevier Excerpta Medica - North-Holland, 1976.

166. Stein, L., Beraud, J-J., Morissette, M. et al: Pulmonary Edema During Volume Infusion. Circ. 52:483, 1975.

167. Stossel, T.P.: Phagocytosis (First of three parts). New Eng. J. of Med. 290:717, 1974.

168. Szidon, P., Bairey, N., Oparil, S.: Effect of Acute Hypoxia on the Pulmonary Conversion of Angiotensin I to Angiotensin II in Dogs. Circ. Res. 46:221, 1980.

169. Tanner, A.R., Halliday, J.W., Powell, L.W.: Effect of Long-term Corticosteroid Therapy on Monocyte Chemotaxis in Man. Scand. J. Immunol. 11:335, 1980.

170. Taylor, A.E., Gaar, Jr., K.A.: Estimation of Equivalent Pore Radii of Pulmonary Capillary and Alveolar Membranes. Am. J. Physiol. 218:1133, 1970.

171. Terry, P.B., Traystman, R.J., Newball, H.H. et al: Collateral Ventilation in Man. New Eng. J. of Med. 298:10, 1978.

172. Thomas, L.: The lives of a Cell - Notes of a Biology Watcher. Bantam Books Inc., New York, 1974.

173. Thrall, R.S., Peterson, L.B., Braley, J.F. et al: Chronic Pulmonary Inflammation Modulates the Fate of Proteins Administered by the Respiratory Tract. J. Lab. Clin. Med. 95:343, 1980.

174. Toews, G.B., Gross, G.N. Pierce, A.K.: The Relationship of Inoculum Size to Lung Bacterial Clearance and Phagocytic Cell Response in Mice. Am. Rev. of Resp. Dis. 120:559, 1979.

175. Tono-Oka, T., Nakayama, M., Matsumoto, S.: Human Monocyte-derived Chemotactic Factor for Granulocytes. Immunology, 39:607, 1980.

176. Vaughan, Jr., Th.R., Erdmann, III, A.J., Brigham, K.L. et al. Equilibration of Intravascular Albumin with Lung Lymph in Unanesthetized Sheep. Lymphology, 12: 217, 1979.

177. Vreim, C.E., Ohkuda, K.: Improved Method for Cannulation of the Right Lymph Duct in Dogs. J. Appl. Physiol. 43:899, 1977.

178. Vreim, C.E., Ohkuda, K., Staub, N.C.: Proportions of Dog Lung Lymph in the Thoracic and Right Lymph Ducts. J. Appl. Physiol. 43:894, 1977.

179. Wangensteen, D., Yankovich, R.: Alveolar Epithelium Transport of Albumin and Sucrose: Concentration Difference Effect. J. Appl. Physiol. 47:846, 1979.

180. Whaley, K.: Biosynthesis of the Complement Components and the Regulatory Proteins of the Alternative Complement Pathway by Human Peripheral Blood Monocytes. J. Exp. Med. 151:501, 1980.

181. White, J.R., Froeb, H.F.: Small-Airways Dysfunction in Nonsmokers Chronically Exposed to Tobacco Smoke. New Eng. J. of Med. 302:720, 1980.

182. Winlove, C.P., Davis, J., Rubin, B.T. et al: The use of Radioiodinated Human Serum Albumin in Measurements of Arterial Permeability. Cardiovasc. Res. 12:609, 1978.

183. Woolverton, W.C., Brigham, K.L, Staub, N.C.: Effect of Positive Pressure Breathing on Lung Lymph Flow and Water Content in Sheep. Circ. Res. 42:550, 1978.

184. Zapol, W.M., Snider, M.T.: Pulmonary Hypertension in Severe Acute Respiratory Failure. New Eng. J. of Med. 296:476, 1977.

185. Yipintsoi, T., Hagstrom, J.W.C., Veith, F.J.: Pulmonary Capillary Permeability to Sodium in Unilateral Transplanted Canine Lungs. J. of Surg. Res. 27:353, 1979.