1973

Use of the nitroblue tetrazolium dye test as an aid in managing patients with cystic fibrosis

James Francis Sullivan
Yale University

Follow this and additional works at: http://elischolar.library.yale.edu/ymtdl

Recommended Citation
http://elischolar.library.yale.edu/ymtdl/3220

This Open Access Thesis is brought to you for free and open access by the School of Medicine at EliScholar – A Digital Platform for Scholarly Publishing at Yale. It has been accepted for inclusion in Yale Medicine Thesis Digital Library by an authorized administrator of EliScholar – A Digital Platform for Scholarly Publishing at Yale. For more information, please contact elischolar@yale.edu.
USE OF THE NITROBLUE TETRAZOLIUM DYE TEST
AS AN AID IN MANAGING PATIENTS
WITH CYSTIC FIBROSIS

JAMES FRANCIS SULLIVAN

1973
Permission for photocopying or microfilming of "Use of the Tetracycline Dye Test as an Aid in Managing Patients with Cystic Fibrosis" (TITLE OF THESIS) for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

[Signature of Author]

[5/9/73]

Date
USE OF THE NITROBLUE TETRAZOLIUM
DYE TEST AS AN AID IN MANAGING
PATIENTS WITH CYSTIC FIBROSIS

James Francis Sullivan
B.A. Manhattan College, 1969

A Thesis Submitted in Partial Fulfillment
of the Requirements for the
Degree of
Doctor of Medicine

Yale University School of Medicine
1973
Acknowledgements

The author would like to express his gratitude and appreciation to the following individuals:

- To my advisors, Dr. Thomas F. Dolan, Jr., and Dr. Robert H. Gifford for their friendly encouragement, advice and criticism during the preparation of my thesis and for the opportunity to work in their laboratories. Their friendship made the preparation of this thesis a worthwhile experience.

- To Mrs. Kathleen Treat for expert technical assistance.

- To Mrs. Dorothea McManamy and Miss Nancy Schwab for fine secretarial assistance.
# Table of Contents

**Introduction**  
NBT False Positive Results  
NBT False Negative Reactions  
Chronic Granulomatous Disease, Phagocytosis, and Intracellular Bactericidal Capacity  
Theories on the Mechanism of NBT Dye Reduction  
Cystic Fibrosis  
Application of the NBT Dye Test to Cystic Fibrosis: Experimental Proposals  
Methods and Materials  
Results  
Discussion  
Summary  
Tables and Figures  
Bibliography
Introduction

The white blood cell count is frequently used as a major tool in the differential diagnosis of infectious diseases. Although leukocytosis with a shift to the left is often indicative of bacterial infection, it is also known to accompany such stressful situations as diabetic ketoacidosis, burns, strenuous exercise, seizures, serum sickness, paroxysmal tachycardia, salicylate or hydrocarbon poisoning, ether anesthesia, the postoperative state, immunizations, hemolysis, and serious hemorrhage.

In his pioneer studies on the enzyme leukocyte alkaline phosphatase (LAP), Wachstein discovered that the amount of LAP per neutrophil and the number of LAP(+) neutrophils increased with bacterial infection and he postulated that the increased enzyme activity in neutrophils under inflammatory stimuli probably signified increased cell metabolism. Ruttenburg demonstrated increased LAP activity not only with bacterial infection, but also in pregnancy, leukemoid reactions, and some cases of myeloid metaplasia and polycythemia vera. Beisel discovered that LAP rises with some viral and rickettsial infections and that the rises in LAP with bacterial or viral infection are often variable in onset, sometime occurring several days after the fever and leukocytosis have disappeared. Thus, changes in LAP have proved to be of little value in the diagnosis of bacterial infection.

A major step in the differentiation of bacterial infection from those non-bacterial illnesses often confused with bacterial
infection because of fever, leukocytosis, and other clinical symptoms has been made by Park and his associates with the use of the nitroblue tetrazolium (NBT) dye test. These investigators demonstrated that there is a significant difference in the percentage of neutrophils reducing the dye to an intracellular blue-black formazan precipitate (NBT(+) neutrophils) in patients with active bacterial infection (mean 29-47%) vs. patients with viral illnesses or other noninfectious fevers (including tuberculosis and collagen diseases) and normal controls (mean 5.8-9.5%). Their results were confirmed in a larger series of patients by Feigin et al. in children and Matula and Paterson in adults as well as in smaller series by other investigators. In addition, Feigin's group, using discriminant analysis of the percentage and absolute number of NBT (+) cells, were able to construct a nomogram dividing their 349 febrile patients into 4 groups: 1) normal subjects, 2) individuals with viral infection, noninfectious fevers, or partially treated bacterial infection, 3) untreated bacterial infections and 4) bacterial infection unresponsive to the therapy provided. In my thesis, I have applied the NBT dye test to patients with cystic fibrosis in whom the detection of active bacterial infection is often difficult. My experimental proposals will be discussed later in the paper.

**NBT False Positive Results**

Despite significant support for the efficacy of the NBT test in the differential diagnosis of bacterial from non-bacterial
disease, there have been cases of false positive and false negative results.

A number of false positive NBT results have been reported in the literature:

A) **Parasitic Diseases** - NBT results in the range usually found in active bacterial infection (greater than 10% NBT (+) neutrophils according to Matula and Paterson) have been found in thirteen patients with malaria, in one patient each with loiasis, trichinosis, and amebic liver abscess and in three dogs infected with *Dirofilaria immitis* (dog heartworm).

B) **Fungal Diseases** - high NBT values have been reported in four patients with *Candida albicans* septicemia and in two patients with pulmonary nocardiosis.

C) **Viral and Mycoplasma Infections** - Humbert reported high NBT results in five out of twenty-five patients with viral central nervous system infections (all five suspected or proven Echovirus infections) while Elgefors reported elevated NBT results in four out of eleven patients with viral meningitis - one Coxsackie, two Herpes and one mumps meningitis. Freeman recently demonstrated high NBT results in two patients with Mycoplasma pneumonia in which seroconversion was demonstrated.

D) **Newborn and Premature Infants** - Park et al., Humbert et al., and Wehinger have demonstrated elevated NBT responses in healthy term infants using the method of Park et al. (Park, Humbert), the method of Gifford and Malawista.
(Wehinger) and a quantitative determination of NBT reduction developed by Baehner and Nathan (Humbert). The elevated NBT responses are said to return to normal levels in 7 - 20 days or within two months. Park, Holmes and Good have demonstrated an increased oxygen consumption, an increased hexose monophosphate shunt activity and an increased quantitative NBT reduction in the resting state of newborn infants and they interpret the "false" positive NBT values as a result of increased metabolic activity in the leukocyte.

Elevated NBT values were also found in the neutrophils of healthy premature infants, and those with non-bacterial disease by Cocchi et al. who, in addition, found a decrease in NBT reduction to "normal" levels (less than 10%) in premature infants with bacterial infection.

E) Miscellaneous - False-positive NBT reduction reactions have also been reported in Chediak-Higashi disease, after typhoid-paratyphoid vaccine, in a case of suspected drug overdosage without infection, in Hodgkin's disease with fever, in myelofibrosis, and in four out of six patients with hemophilia by Humbert et al. Humbert et al. also reported elevated NBT reduction, leukocyte respiration, and hexose monophosphate shunt activity in several patients with ostenogenesis imperfecta, but these results are disputed by Brown et al. The concentration of heparin used in the method of Park et al has been reported to be a critical factor.
determining the ranges of "normal" and has been reported to elevate the level of spontaneous NBT reduction by neutrophils. The critical role of heparin concentration has also been noted in several of my experiments.

**NBT False Negative Reactions**

**A) Bacterial Infections** - Esposito and Lalla reported twelve cases of bacterial meningitis in which the peripheral blood neutrophils showed an NBT reduction response within the normal range although the neutrophils within the cerebrospinal fluid (CSF) showed a strongly positive NBT response. This is in contrast to positive NBT reduction findings in peripheral blood neutrophils in bacterial meningitis reported by Park et al., Feigin et al., Matula and Paterson, and Humbert et al.

Ng reported three out of seven patients with subacute bacterial endocarditis (SBE) had NBT reduction values by neutrophils within the normal range in contrast to high NBT reduction values reported by Park et al.

Localized infections can also occasionally give negative NBT responses as is the case with four patients reported by Matula and Paterson with: staphylococcal osteomyelitis (presumably after the hematogenous phase); cellulitis due to Group A beta-hemolytic streptococci; urethritis-cystitis due to E. coli; and an enterococcal pelvic abscess.

Miscellaneous bacterial infections in which the NBT
response has been negative included a patient with nephrosis and pneumococcal peritonitis cited by Park and three cases of infected ventriculo-peritoneal shunts reported by Humbert et al.

B) Drugs - Strauss et al. found that phenylbutazone inhibited both the engulfment and intracellular killing of E. coli by guinea pig polymorphonuclear leukocytes and also decreased the quantitative reduction of NBT by neutrophils stimulated with latex particles while Klebanoff and White found that methimazole inhibited the killing of Lactobacillus acidophilus by the leukocytes of a patient with chronic granulomatous disease and the patient's mother's, but not by normal leukocytes.

The effect of steroids on the NBT test is controversial. Miller and Kaplan report that the quantitative NBT dye reduction was decreased in twenty-one children receiving prednisone in a dose of 1.0-2.0 mg/kg. compared to normal controls. Matula and Paterson report one adult patient on 1 gram of methylprednisolone and 150-200 mg. azathioprine daily with peritonitis and multiple pelvic abscesses in which the spontaneous NBT dye reduction by neutrophils was negative, but the "stimulated" NBT test using 200 ug/ml of bacterial endotoxin described by Park and Good was positive. Ng reported low NBT reduction and low "stimulated" NBT tests with an undisclosed number of patients on steroids. In contrast to these results, Matula and Paterson, in another paper, describe nine adult patients receiving the equivalent of
20-120 mg. of prednisone daily in which the NBT test was not affected. In addition, a recent paper showed that the NBT test was not affected by large doses of steroids and azathioprine in renal transplant patients.

Similar controversy surrounds the effects of immunosuppressive or cytotoxic drugs. Miller and Kaplan report normal NBT dye reduction in leukemic children receiving 6-mercaptopurine and amethopterin, while Humbert et al. report decreased NBT dye reduction in two patients with septicemia on cytotoxic drugs (histiocytosis X treated with vinblastine and lymphoma treated with nitrogen mustard). Malawista demonstrated that vinblastine does inhibit the increased oxygen consumption and glucose \(-1\,\text{C}\) oxidation that normally accompanies phagocytosis and that it appears to limit lysosomal degranulation even though the ingestion process itself and intracellular killing of bacteria did not appear to be impaired, although a bacterial killing defect was postulated in an earlier paper.

Sulfonamides and certain other anti-inflammatory drugs found to be myeloperoxidase (an enzyme important in leukocyte bactericidal activity) inhibitors may also result in a false negative NBT test but apparently no patients have been tested as yet.

C) Neutrophil metabolic defects, inherited or acquired -

The major area where false negative NBT dye reduction tests occur is with neutrophil metabolic defects or with some impairment in host humoral or cellular defense mechanisms.
1) Inherited - The classic host defense disease with a neutrophil defect in which the NBT dye test is negative is chronic granulomatous disease of childhood. The importance of this disease in the development of the NBT test and in the elucidation of the mechanism of phagocytosis and intracellular killing of bacteria will be discussed in the next section. One patient with hereditary myeloperoxidase deficiency and a patient with complete deficiency of glucose-6-phosphate dehydrogenase demonstrated defective bactericidal capacity and a failure to reduce NBT. In addition, congenital agammaglobulinemia has been reported to give negative results. A patient with sickle cell disease and Salmonella osteomyelitis has been reported to have a false negative NBT dye reduction test, presumably due to defective polymorphonuclear leukocyte function in this disease.

2) Acquired - Impaired neutrophil bactericidal capacity was demonstrated with five test organisms and a decreased quantitative NBT dye reduction test in a 75 year old patient with mixed cryoglobulinemia by Douglas et al., an acquired and apparently transient, reversible condition. Certain of the false negative NBT reduction group reported by Humbert et al. were postulated to have altered WBC function that may have affected the results: two alcoholics, one with Pseudomonas aeruginosa brain abscesses and one with Streptococcus pyogenes.
subdural empyema; a patient with severe burns and *Pseudomonas* and *Candida albicans* septicemia; and one case of pneumonia complicating an influenza syndrome. Brayton *et al.* have shown that alcohol depresses the rate of leukocyte mobilization into traumatized skin of nutritionally normal people but human polymorphonuclear leukocytes obtained after alcohol was given or leukocytes exposed to alcohol *in vitro* showed no decrease in ability to ingest or kill bacteria. This observation plus the fact that no NBT dye reduction tests have been performed in leukocytes exposed to alcohol must lead one to doubt alcohol as a cause of the two false negative results in infected alcoholics. Thermal burns have been shown to affect neutrophil function (phagocytosis and intracellular killing) and influenza virus has been shown to reduce the phagocytosis of pneumococci by exudate polymorphonuclear leukocytes, macrophages, and alveolar macrophages in experimental animals. Both could plausibly affect WBC function and thus account for false negative NBT results.

**Chronic Granulomatous Disease, Phagocytosis, and Intracellular Bactericidal Capacity**

In 1957 Berendes, Bridges, and Good characterized the features of a new syndrome, also elaborated by Landing and Shirkey, which they later named fatal (chronic) granulomatous disease of childhood. The syndrome consisted of lymphadenitis,
hepato-splenomegaly, and pulmonary infiltrates, pathologically
distinguished by granulomas which were clearly different from
other infectious and non-infectious granulomatous processes.
Patients reported with this syndrome have usually been males
with the onset of severe bacterial disease early in life.
Polymorphonuclear (PMN) leukocytes of patients with chronic
granulomatous disease of childhood (CGD) were noted to
phagocytize bacteria normally but to permit intracellular
survival of certain bacteria, along with a failure to reduce
NBT dye. These basic findings in PMN leukocytes of patients
with CGD stimulated work which elaborated not only the basic
features of this rare disease, but also some of the basic meta¬
bolic events associated with phagocytosis and intracellular
reduction and its intimate tie-in with these events.

The mechanisms of bacterial killing by polymorphonuclear
leukocytes are not completely understood. Phagocytosis of
bacteria is associated with the rupture of leukocyte granules
(lysosomes) and the discharge of their contents into the phago¬
cytic vacuole containing the ingested organisms. Leukocytic
granules contain a variety of antibacterial agents among which
are lysozyme, a number of granular cationic proteins and
myeloperoxidase. There is also a burst of leukocyte metabolic
activity after phagocytosis that results in a sharp fall in
pH in the vicinity of the ingested particle, and in the generation
of hydrogen peroxide by the cell. Normally, these events are accompanied by the death of most organisms. Quie et al demonstrated that the total lysozyme and phagocytin activity of leukocyte extracts from patients with CGD were similar to control leukocytes and they postulated that the deficiency of bactericidal capacity of these leukocytes was due to the minimal degranulation of lysosomes which they noted after phagocytosis. Further studies demonstrated that PMN leukocytes of patients with CGD responded to the phagocytosis of latex particles with normal increments in glucose consumption, lactate production, Krebs cycle activity, and lipid activity as do PMN leukocytes from normal patients. These events were postulated to be associated with the phagocytic process, a logical deduction since phagocytosis is normal in both CGD and control leukocytes. However, unlike normal leukocytes, the leukocytes of these patients fail to show normal increments in respiration, direct oxidation of glucose through the hexose monophosphate (HMP) shunt, and hydrogen peroxide formation during particle uptake, events thought to be associated with lysosomal degranulation and intracellular bactericidal capacity. In normal leukocytes, glycolytic inhibitors such as iodoacetate and fluoride inhibit phagocytosis whereas respiratory inhibitors such as cyanide and actinomycin A do not, indicating that the energy required for engulfment comes from glycolysis. In addition, methylene blue equally stimulated HMP activity in control and CGD leukocytes.
Therefore, the HMP pathway is present in CGD leukocytes, but is not stimulated by phagocytosis.

A unified concept relating the various metabolic abnormalities found in CGD has centered around the deficiency of H\textsubscript{2}O\textsubscript{2} in the leukocytes of these patients. Leukocytes of patients with CGD can kill organisms which produce their own H\textsubscript{2}O\textsubscript{2} such as streptococci, (catalase negative) but they cannot kill organisms which produce catalase to destroy H\textsubscript{2}O\textsubscript{2} like Staphylococcus or Serratia. Lehrer has demonstrated improved candidacidal activity in CGD leukocytes with the use of the H\textsubscript{2}O\textsubscript{2} producing drug, phenazine. In addition, Reed has implicated H\textsubscript{2}O\textsubscript{2} in the stimulation of the HMP shunt and thus in CGD the deficiency of H\textsubscript{2}O\textsubscript{2} might also be responsible for the lack of stimulation of the HMP shunt. Klebanoff and McRipley and Sbarra demonstrated the bactericidal potential of myeloperoxidase, iodine and H\textsubscript{2}O\textsubscript{2} in the presence of bacteria, thus demonstrating a major pathway in which H\textsubscript{2}O\textsubscript{2} is used to kill bacteria intracellularly.

Klebanoff and White demonstrated that neutrophils from a patient with CGD, unlike normal neutrophils, did not fix iodine after the ingestion of heat killed Lactobacillus acidophilus or heat killed or live Serratia marcescens. When live L. acidophilus was employed (produces H\textsubscript{2}O\textsubscript{2}), iodination did occur, although it was less than normal.

Baehner and Nathan and Baehner and Karnovsky have found a deficiency of a cyanide-insensitive NADH oxidase in the leukocytes...
of patients with CGD, a finding they believe can account for the metabolic abnormalities in their leukocytes. The postulated mechanism is as follows:

\[
2 \text{NADH} + 2\text{H}^+ + 2\text{O}_2 → \text{NAD}^+ + 2\text{H}_2\text{O}_2
\]

\[
2 \text{H}_2\text{O}_2 + 4 \text{GSH} \rightarrow 2 \text{GSSG} + 4 \text{H}_2\text{O} \quad (\text{reduced glutathione overoxidase})
\]

\[
2 \text{GSSG} + 2 \text{NADPH} + 2\text{H}^+ → 2 \text{NADP}^+ + 4 \text{GSH} \quad (\text{glutathione reductase})
\]

A deficiency of NADH oxidase would thus account for a lack of \(\text{H}_2\text{O}_2\) and indirectly, since \(\text{NADP}^+\) is a factor known to control HMP pathway activity, account for the lack of stimulation of HMP shunt activity in the leukocytes of these patients. This finding is supported by Cagan and Karnovsky who demonstrated that the respiratory burst during phagocytosis in guinea pig polymorphonuclear (PMN) leukocytes results from the action of a cyanide-insensitive NADH oxidase, which produces \(\text{H}_2\text{O}_2\). Further substantiation of this postulate was provided by Baehner, Nathan, and Karnovsky who inserted an \(\text{H}_2\text{O}_2\) generating system into CGD leukocytes by allowing the cells to phagocytize latex spherules coated with glucose oxidase. This produced amelioration in the known metabolic deficiencies of these cells during phagocytosis: a) formate oxidation dependent of \(\text{H}_2\text{O}_2\) production increased four times b) HMP shunt activity was markedly stimulated and c) there was an increase towards normal levels.
of NBT dye reduction of CGD leukocytes. However, these results are contradicted by Holmes, Page and Good, who found that the NADH oxidase activity of CGD leukocytes was equal to that of normal control leukocytes.

Previously, I had mentioned that CGD is thought to be primarily a sex-linked recessive found most often in males. Evidence for this is provided by Windhorst, Holmes and Good who found two populations of cells in PMN leukocytes of carrier females. Leukocytes of all eight mothers and several sisters of affected male patients were intermediate in bactericidal capacity and quantitative NBT dye reduction between leukocytes of affected boys and controls in line with the Lyon hypothesis. This result has been verified by others. However, a few cases of CGD have been reported in female children in which no clinical or laboratory evidence of a leukocyte abnormality (including normal NBT dye reduction) could be demonstrated in family members, prompting an hypothesis that these cases could be instances of autosomal recessive inheritance. Interestingly, Holmes et.al. found that leukocyte glutathione peroxidase activity was significantly diminished in two female patients with CGD as compared to control leukocytes. By contrast, leukocytes of male patients with CGD showed normal activity of this enzyme. To support their position, Holmes et.al. have cited the following postulated reactions to account for the metabolic deficiencies of CGD leukocytes in their female patients with glutathione peroxidase deficiency:
In their scheme, a limitation in the pathway due to a deficiency of glutathione peroxidase would block the cycle by limiting the availability of GSSG (oxidized glutathione), the substrate for glutathione reductase. Normal stimulation of the HMP pathway would not occur, and oxygen consumption and hydrogen peroxide production would be blocked owing to a lack of NADPH.

Thus two enzyme defects are postulated in CGD; an NADH oxidase defect reported in both male and female CGD patients and a glutathione peroxidase defect reported in certain female but not male patients with CGD, each presented with theoretical reactions which could account for the lack of \( \text{H}_2\text{O}_2 \) production and the lack of stimulation of HMP shunt activity in the leukocytes of these patients thus accounting for the bactericidal defect and possibly for the mechanism of NBT reduction.

**Theories on the Mechanism of NBT Dye Reduction**

The major theory concerning the mechanism of increased NBT dye reduction during bacterial infection assumes: 1) that the phagocytosis system of the host, both cellular and humoral, is operating normally and 2) that the infection involves the
systemic circulation. This idea of the NBT test is based on
the hypothesis that a metabolic change and an increased reduction
of NBT dye would take place when leukocytes are involved in vivo
during a natural infection. Oxidized (yellow) NBT dye in solution
does not penetrate cell membranes well, since it is positively
charged, therefore it is presumed that the NBT has to enter
the cell attached to a phagocytized particle or in the fluid
around it. The theory is that NBT dye reduction is increased
in bacterial infection as opposed to normal controls or nonin-
fectious fevers because contact with bacteria or bacterial
products stimulates phagocytosis and thus ingestion of the dye.

The increased intracellular metabolism within the polymorphonuclear
leukocyte, which is stimulated by phagocytosis, presumably is
also associated with the increased reduction of the oxidized,
yellow NBT dye to a reduced, blue-black formazan precipitate.

Baehner and Nathan, from their studies of CGD leukocytes, feel
that NADH oxidase is the intracellular enzyme responsible for
NBT dye reduction in the PMN leukocyte. The importance of
phagocytosis can be illustrated by the method of Gifford and
Malawista. Isolating granulocytes by allowing them to adhere
to a glass surface, these investigators found a high NBT re-
duction in normal granulocytes. In the presence of infection
the percentage of NBT (+) cells did not increase, presumably
since the granulocytes were already "phagocytizing" glass and
thus maximally stimulated.
There is also experimental support that contact with bacteria or bacterial products is responsible for the increased NBT dye reduction during bacterial infection. Cocchi demonstrated increased NBT dye reduction when polys were incubated with Pseudomonas. Park and Good and Matula and Paterson also showed stimulation of NBT dye reduction when the PMN leukocytes were incubated with endotoxin. Staphylococcal protein-A and streptolysin-O can also induce an increased reduction of NBT dye when they are added to whole blood, thus it is assumed that any bacterial product or bacterial component may also be responsible for the increased NBT dye reduction with bacterial infection by stimulating increased phagocytosis and intracellular reduction of the dye.

Crush feels that contact with bacteria or particulate matter to stimulate the PMN leukocytes does not have to be postulated and that lysosomal instability presumably can just be the cause of increased NBT reduction.

Gifford and Malawista, in examining NBT dye reduction by their method under different experimental conditions, concluded that the permeability of the granulocyte to NBT may be the critical variant and can be altered by changes in NBT concentration, particle ingestion, and adherence to a surface. They postulated that reports which describe an increase or decrease in NBT reduction associated with certain clinical disorders need not be interpreted to represent a stimulation or

17
depression of the enzyme system(s) necessary for NBT reduction (or bactericidal capacity) as in CGD, but may be due, at least in part, to change in cell permeability to NBT.

**Cystic Fibrosis**

Cystic Fibrosis is a hereditary disease of children, adolescents, and young adults in which there is a generalized dysfunction of exocrine glands. The triad of chronic pulmonary disease, pancreatic insufficiency and abnormally high sweat electrolytes is present in most patients. Hepatic cirrhosis, intestinal obstruction edema, malabsorption, nasal polyps, rectal prolapse and many other manifestations may be part of the clinical picture of this protean disease. Most of these symptoms are thought to be secondary to obstruction of the organ passages (such as bronchioles, pancreatic ducts and biliary ductules). There is general agreement that C.F. is due to an inborn error of metabolism transmitted as an autosomal recessive trait with an incidence of about 1 in 2000 live births in countries with populations predominantly of Caucasian descent. The etiology of the disease is unknown, but many studies have focused on the abnormally viscid mucus as the key to understanding this disease. It has been suggested by Gibson, Matthews and Minihan that a primary biological function of mucus is to protect cell membranes from hydrostatic, osmotic, and concentration gradients. The consequences predicted
for an impairment of this function (which would make mucus "leaky" or "hyperpermeable") fit in with most of the known characteristics of exocrine secretions from patients with cystic fibrosis. Cystic fibrosis (C.F.) mucus was shown to be hyperpermeable in vitro and serum from patients with C.F. can induce hyperpermeability in vivo. Apart from its viscosity, the most definitive abnormality of C.F. mucus is the quantitative, but not qualitative difference between the electrophoretic patterns produced by C.F. and normal submaxillary glycoprotein. This difference seems entirely due to calcium since the addition of calcium to normal mucus produces a C.F. pattern, while the removal of calcium from C.F. mucus with edetic acid produces a normal pattern. This is especially interesting since probably all C.F. exocrine secretions contain excess calcium. In the studies by Gibson, Matthews, and Minihan, calcium was found to increase mucus permeability in vitro and in vivo and, when iontophoresed into the skin, to raise the salt concentration of sweat. They postulated that C.F. patients may have normal mucus, but secrete an excess amount of calcium which combines with the mucus and renders it hyperpermeable.

Application of the NBT Dye Test to Cystic Fibrosis: Experimental Proposals

As was mentioned previously, C.F. patients are prone to numerous pulmonary infections and it is usually respiratory insufficiency secondary to chronic pulmonary disease that is the
cause of their demise. Infection developing distal to mucus plugs in bronchioles is the most likely explanation for their frequent infections, but a defect in these patients' polymorphonuclear leukocytes has not yet been ruled out. It is difficult to assess the presence or absence of acute bacterial infection in these patients since pathogens are frequently grown from the sputum of even asymptomatic patients and other clinical parameters may be non-contributory. In addition, even if antibiotic therapy is started for a presumed flareup of lung disease, its effectiveness is difficult to assess since often appropriate antibiotics may not change the patient's sputum flora.

Since the recently introduced NBT dye reduction test has been shown to be a useful guide in the diagnosis and management of bacterial infections, I decided to use the NBT test in order to determine:

1) whether there was any difference in NBT dye reduction by leukocytes of C.F. patients as compared to normal controls as one method of determining the presence or absence of a bactericidal defect in polymorphonuclear leukocytes of C.F. patients.

2) whether the NBT test could be used as a useful parameter in determining the presence or absence of an acute bacterial infection in these patients.
3) whether the NBT test would be a useful guide to assess the effectiveness of antibiotic therapy in these patients.

Other clinical parameters such as white blood cell count, band shift, temperature, physical findings, sputum cultures and chest X-ray results were also examined to determine their effectiveness in predicting the presence or absence of bacterial infection.
Methods and Materials

Patients

157 nitroblue tetrazolium (NBT) dye tests were performed on heparinized venous blood obtained from 78 cystic fibrosis (C.F.) patients seen at Yale-New Haven Hospital during the period December 1971-May 1972. The diagnosis of cystic fibrosis was established in these patients by means of elevated sweat chloride determinations and a typical clinical picture. The patients ranged in age from 7 months to 26 1/2 years with a mean of 8.6 years.

At each visit, the patients were placed into one of five groups on the basis of the Shwachman Chest X-ray score (see Table 8) and the clinical assessment of the senior physician on the basis of history, physical examination, chest x-ray, and other laboratory parameters if available. The results of the NBT dye test were not known at the time the categorization was made and the clinical assessment was not known to the person reading the results of the NBT test.

The groupings were as follows: Group I patients had Shwachman Chest X-ray scores greater than 19 and were stable and doing well. Group II patients also had Shwachman Chest X-ray scores greater than 19 but were thought to have an acute flareup of their disease with an increase in respiratory symptomatology and a pulmonic process thought to be bacterial. Group III
patients had more chronic changes in their lungs with Shwachman Chest X-ray scores less than 19 but were thought to be stable and doing well, on or off antibiotics. Group IV patients had Shwachman Chest X-ray scores less than 19 but were thought to have an acute flareup of their disease with an increase in respiratory symptomatology and a pulmonic process thought to be bacterial. Group V patients were a small, difficult group to clinically define with Shwachman Chest X-ray scores less than 19 who were thought to be chronically infected and going gradually downhill without ever going back to a stable baseline - continuously doing poorly with exercise intolerance, dyspnea or tachypnea, bouts of hemoptysis, copious sputum production, poor pulmonary function tests, with rales and rhonchi consistently heard in their chest and on continuous antibiotic therapy without amelioration of symptoms.

I also analyzed my results by dividing the patients into those judged clinically well (Groups I and III), those judged clinically sick (Groups II and IV) and those judged to be chronically infected (Group V).

I also compared the results of the NBT test with other clinical parameters - white blood cell count, band shift (shift to the left in the polymorphonuclear (PMN) leukocyte series), temperature, the presence or absence of rales, sputum culture results and findings on chest x-ray to see how good an indicator
each might be in predicting the presence or absence of a bacterial infection.

**Laboratory Methods**

The NBT dye test was performed according to the method of Park *et al* with a slight modification. One cc of venous blood was drawn into a plastic syringe and transferred to a disposable plastic tube to which 100 units of heparin were added. At the same time, blood was drawn for a white blood cell count and differential count to be performed by the Yale-New Haven Hospital Hematology Lab, or, in certain instances, by myself. Approximately 0.1 ml. of this heparinized blood was then transferred into a clean, concave microslide (Macalaster-Bicknell Co., New Haven, Conn.) and mixed with an equal amount of NBT solution (a mixture of equal amounts of 0.2% NBT (Nutritional Biochemicals Corp., Cleveland) in physiological saline solution and 0.15 M phosphate-buffered saline solution - pH 7.2). The slides were placed in petri dishes containing wet gauze to provide humidity and were then incubated at 37°C. for 15 minutes and subsequently kept at room temperature for an additional 15 minutes. At the end of this period, the blood-NBT mixture was again mixed using a small wooden applicator stick. Coverslip smears were carefully made, air dried, and counterstained with Wright's stain. The tests were all performed within 1/2 hour of obtaining the blood samples.
Cell Count

Wright stained smears were examined under the microscope with the high, dry objective (450X). Initially three separate counts of 100 neutrophils each was performed on each test but since a good correlation was obtained on each of the counts, only 100 neutrophils were counted for the majority of tests. Monocytes were not counted. Only those cells which were intact and could be clearly identified as neutrophils with a discrete deposit of blue-black formazan were classified at NBT positive. The intracellular formazan deposit could be either in the form of a "clump" or a "streak" of blue-black granules. The absolute number of positive neutrophils per cu. mm. was calculated using the white blood cell count, differential, and the percentage of NBT (+) neutrophils. In our study, as well as others, NBT scores above 10% were considered abnormal, or NBT positive, correlating with the presence of active bacterial infection. NBT scores below 10% were considered to be in the normal range, but were also seen in patients with viral disease or other non-infectious febrile disease.
Results

131 tests on 78 separate patients with cystic fibrosis were classified into one of five groups according to the criteria discussed earlier. Twenty-six tests were not classified into one of the five groups because they were part of sequential studies taken while the patients were on antibiotics for a presumed flareup of respiratory infection and will be discussed separately.

Table 1 shows the results of the one hundred thirty-one tests broken down according to the five groups. The results are also analyzed as to the number of tests in each group that had less than or equal to 10% of the neutrophils reducing the dye and those that had greater than 10% of the neutrophils reducing the dye. The results of my scatter plot of values (Figure 2) indicated that 10% NBT (+) neutrophils would be a good cutoff point in my series. Patients placed in Group I had an age range from 2-19 1/2 years and had a mean percentage of NBT (+) neutrophils of 7.4 + 5.1% with a range of 1-32%. Thirty-eight tests had an NBT score less than or equal to 10% and four had an NBT score greater than 10% which is statistically significant (p < .001). Of the four patients with NBT scores > 10%, two values (11% and 32%) were obtained on two separate occasions from one patient thought to be clinically healthy; another value of 11% on a clinically healthy patient and a value of 20% on a patient thought to be stable at the time of his clinic visit but who spiked a temperature to 102°F.
that night with increased cough and sputum production.

Patients placed in Group II with a presumed flareup of respiratory infection had an age range from 7 months to 18 years and had a mean percentage of NBT (+) neutrophils of 23.1 ± 11.3% with a range of 9-56%. Eighteen tests had an NBT score of greater than 10% and one test (9%) had an NBT score less than or equal to 10%, which is statistically significant (p<.001).

Patients placed into Group III had an age range from 1-18 years with a mean percentage of NBT (+) neutrophils of 6.3 ±2.5% with a range of 2-14%. Forty tests had an NBT score of <10% and two tests (11% and 14% on two separate patients) had an NBT score of greater than 10% which is statistically significant (p<.001).

Group IV patients with a presumed flareup of respiratory infection had an age range from 3-26 1/2 years with a mean percentage of NBT (+) neutrophils of 17.4 ± 5.6% with a range of 4-27%. Nineteen tests had an NBT score of greater than 10% and two tests (8% and 4% on two separate patients) had an NBT score of ≤10%, which is statistically significant (p<.001).

Group V contained a small number of patients with an age range from 5-19 1/2 years with a mean percentage of NBT (+) neutrophils of 9.4 ± 3.4% with a range of 5-15%. Five tests had an NBT score of ≤10% and two tests had an NBT score greater
than 10%, which was not significant.

Table 2 analyzes the results of the NBT percentage breakdown according to whether the patient was thought to be clinically infected (Groups II and IV), clinically stable (Groups I and III), or chronically infected (Group V). In those patients thought to be clinically stable (84 tests on 62 patients), seventy-eight tests (93%) had an NBT score of \( \leq 10\% \) and six tests (7%) had an NBT score greater than 10%, which is statistically significant \( (p < 0.001) \). In those patients thought to be clinically infected (40 tests on 34 patients), thirty-seven tests (93%) had an NBT score of greater than 10% and three (7%) had an NBT score of \( \leq 10\% \), which is statistically significant \( (p < 0.001) \). Five tests in those patients thought to be chronically infected had an NBT score of \( \leq 10\% \) and two tests had an NBT score of greater than 10%, which is not statistically significant.

Table 3 shows the results of the mean percentage of NBT (+) neutrophils, the mean absolute number of NBT (+) neutrophils, the mean white cell count, the mean percent band forms, the mean temperature and the change in sputum flora in those patients thought to be clinically stable (Groups I and III) and those thought to be clinically infected (Groups II and IV). These same parameters were also analyzed in Table 4 according to the five clinical groups. The mean percentage of NBT (+) neutrophils
based on 84 tests on 62 clinically stable patients was 6.8 ± 4.0% while the mean NBT % in 40 tests on 34 clinically infected patients was 20.1 ± 9.1%, which is a statistically significant difference (p < .01). The mean absolute number of NBT (+) neutrophils is also statistically significant (p < .001) between the two groups, the mean of those clinically stable being 448.2 ± 330.8 and the mean of those thought to be clinically infected of 1412.9 ± 1263.8. The mean white blood cell count (11,500 ± 3,400 vs. 11,500 ± 4400), band shift (2.5 ± 3.6 vs. 4.9 ± 6.7) and temperature (98.8°F ± 0.8 vs. 99.8°F ± 1.5) were not statistically significant between the clinically infected and clinically not infected groups.

Other clinical parameters were analyzed in these patients. Table 5 shows the results of sputum cultures taken on these patients broken down into the type of organism isolated for each of the five groups and whether there was any change in the organism(s) isolated from the most recent culture. Only Group I has a significant number of sputum cultures with normal flora (21 out of 42) while Pseudomonas appears to be the most commonly isolated organism, increasing in proportion as we go from Group I to Group V. Staphylococci and coliform organisms are less frequently isolated and Hemophilus influenza is rarely isolated in our clinic. There was not much difference between the first four groups in the proportion of cultures in which there was a change in the organism(s) isolated from the previous
culture: 20/42 in Group I; 8/19 in Group II; 17/42 in Group III and 9/21 in Group IV. In Group V only one out of seven cultures showed a change in the organism(s) isolated.

Table 6 depicts the presence or absence of a change in the chest x-ray in each of the five groups. In most instances, chest x-rays were not done on that particular day, but in the few that were, a new infiltrate was found in 4 out of 5 films taken in Group II patients but in only 3 out of 9 of the patients in Group IV. In the clinically stable groups, Group I had no films with a new infiltrate and Group III had a change in 4 out of 15 films. In Table 7 rales were found to be present in 9 and absent in 56 of those patients thought to be clinically stable and to be present in 18 and absent in 12 thought to be clinically sick.

Sequential Studies

Fourteen patients have been serially followed with NBT examinations during 16 bouts of presumed bacterial pneumonitis. Prior to their flareup in respiratory symptoms, each patient had a "normal" value of \( \leq 10\% \text{NBT} (+) \) neutrophils. With the onset of the presumed acute bacterial pneumonia, the percentage of NBT (+) neutrophils rose into the "infected" range of greater than 10% in all the patients studied.

In 11 instances of presumed bacterial infection, the institution of antibiotic therapy "appropriate" for the patient's
sputum flora was followed by a prompt resolution in clinical symptomatology and a return of the NBT to "normal" levels. In two instances the NBT test remained elevated and the patients remained symptomatic until an empiric change in antibiotics was instituted, whereupon clinical improvement coincided with a decrease in NBT test to "normal" levels. In three patients, the NBT test did not return to normal and the clinical condition deteriorated in these cases. Figure 1 illustrates the response of the NBT test to antibiotic therapy in two patients, one of whom (pt. B) had a prompt clinical response and a return of the NBT to "normal" levels and one patient (pt. A) just previously mentioned who failed to show a clinical response to antibiotic therapy and eventually expired and whose NBT test remained elevated.
Discussion

Seventy-eight patients with cystic fibrosis of the pancreas were studied prospectively for six months to see if the NBT test would be of value in evaluating these children. The results of the NBT test in these patients provide useful answers to the three experimental proposals previously elaborated:

1) The results of the NBT dye test indicate that the leukocytes of patients with cystic fibrosis reduce the dye in the same relative proportions as do the leukocytes of normal control patients with or without bacterial infection. The mean percentage of neutrophils reducing the NBT dye in our patients with cystic fibrosis who were clinically stable (Groups I & III) was $6.8 \pm 4.0\%$, which is in no way different from normal, healthy controls without cystic fibrosis either in our laboratory ($5.1 \pm 3.6\%$), or as reported by others. The positive NBT tests (mean $20.1 \pm 9.1\%$) in our patients with cystic fibrosis with suspected bacterial infection of the respiratory tract was similar to control patients with bacterial infection both in our lab ($20.0 \pm 6.9\%$) and as reported by others.

2) The results indicate that the NBT test is a very useful parameter in determining the presence or absence of suspected acute bacterial infection in these patients. An NBT test result with $\leq 10\%$ of the patient's neutrophils reducing the dye was correlated in 78 out of 84 instances with a patient
thought to be clinically stable (Groups I & III) while an NBT
test result of $>10\%$ of the patient's neutrophils reducing the
dye was correlated in 37 out of 40 instances with the diagnosis
of probable acute bacterial respiratory infection (Groups II &
IV). It is significant in looking at our six false positive
results that one (20\%) was in a patient thought to be clinically
stable at his clinic visit in the afternoon, but who developed
a temperature of 102°F. with increased cough and sputum production
that night and that our highest false positive value by far (32\%)
was in a girl who had elevated values (11\% and 32\%) on two
separate occasions without any evidence of acute bacterial
disease. The results of the NBT test in our group of chronically
infected patients (Group V) shows a random distribution around
the 10\% cutoff point. This result is not too surprising when
one considers that these patients are more or less continuously
infected and are probably intermittently having acute invasion
of bacteria across bronchial walls. Alternate explanations
include possible metabolic deficiencies in their leukocytes
induced by long-standing disease or by prolonged antibiotic
therapy. The NBT test appears to be particularly helpful in
patients with chest x-ray scores under 19 (Groups III, IV, V)
since this group of patients frequently have Pseudomonas as
the predominant flora (see Table 5), and evidence of an acute
infection requires replacement of an oral antibiotic with
3) Results on a limited number of patients (16 instances of probable bacterial infection in 14 patients) indicate that the NBT test appears to be a useful guide in assessing the effectiveness of antibiotic therapy in patients with cystic fibrosis. In each of the 14 patients a normal baseline NBT value became elevated into the positive range (>10%) with the onset of a probable acute bacterial infection. In 11 instances, the institution of appropriate antibiotic therapy was followed by prompt resolution of the clinical signs and symptoms and was accompanied by a decrease in the NBT test to normal levels. In two instances the NBT test remained elevated and the patients remained symptomatic until a change in antibiotic therapy was instituted, whereupon clinical improvement and a decrease in the NBT results to normal levels occurred. In two instances the patients showed a poor response to antibiotic therapy and their NBT values remained elevated. One patient died and the other was discharged from the hospital with little clinical improvement. Another terminal patient with cystic fibrosis was admitted to the hospital and put on an antibiotic regimen which resulted in the fall in his NBT test to normal levels within three days although he expired later that third day. Whether his therapy was effective in treating his probable bacterial pneumonia and
his death secondary to chronic pulmonary insufficiency with hypoxia and cardiorespiratory arrest or whether this was a false negative result is unknown.

Also significant was my analysis of other clinical parameters usually helpful in the diagnosis of bacterial disease. The mean white blood cell count, the mean percent band forms, the mean temperature and the number of patients with a change in sputum flora from previous culture were not significantly different between those patients thought to be clinically stable (Groups I & III) and those patients (Groups II & IV) thought to be clinically infected (Tables 3 & 4). The presence or absence of rales (Table 7) was not a consistent help in differentiating those thought to be clinically sick from those thought to be clinically stable. In a limited number of studies, the presence or absence of a new infiltrate on chest x-ray (Table 6) was helpful only in differentiating Group I patients (10 out of 10 showed no change on chest x-ray) from Group II patients (4 out of 5 showed a new infiltrate on chest films). The chest x-ray did not appear to be useful in differentiating Groups III, IV, or V most probably because chronic changes in the lungs of these patients tend to obscure the presence of a new infiltrate. Sputum culture results (Table 5) indicate that only Group I has a significant number of cultures with normal flora (21 out of 42) while Pseudomonas appears to be the most commonly
isolated organism, increasing in proportion as we go from Group I to Group V. That Pseudomonas appears to be the most commonly isolated organism in our series, with less isolates of Staphylococcus and coliform organisms and very few isolates of Hemophilus influenza is in disagreement with other studies in which the Staphylococcus is the most common pathogen isolated followed by Pseudomonas, H. influenza and the coliforms. Also significant was the fact that there was not much difference between the first four groups in the proportion of cultures in which there was a change in the organism(s) isolated from the previous study. Thus, the classical rule of isolating the pathogen and treating with appropriate antibiotics is clouded in patients with cystic fibrosis in which acute flareups of bacterial infection may not be reflected by changes in their sputum flora. In addition, the classical rule of treating the infection until the pathogen disappears and clinical improvement occurs also does not completely hold in patients with cystic fibrosis in which appropriate antibiotic therapy may not change the sputum flora (probably because effective concentrations of the antibiotic cannot penetrate into the sputum). In our patients clinical improvement and the return of the NBT test to normal after antibiotic therapy was frequently not accompanied by a change in sputum flora.

In my study, the absolute number of NBT (+) neutrophils was also statistically significant (p < .001) between those patients in Groups I & III thought to be clinically stable.
(448.2 ± 330.8/cu. mm.) and those patients in Groups II & IV thought to be clinically infected (1412.9 ± 1263.8/cu. mm.) (see Table 3). However, in analyzing the scatter plot of values (Figure 2) it was decided that a cutoff of 10% NBT (+) neutrophils would be both a simpler and more reliable test. In addition, a simple analysis of the scatter plot indicates that discriminant analysis using the percentage of NBT (+) neutrophils and the absolute number of NBT (+) neutrophils 29,30 per cu. mm. in the style of Feigin's nomogram 29,30 would not be more beneficial than the simple cutoff of 10% NBT (+) neutrophils. One reason to account for the failure of discriminant analysis to be beneficial in examining our results is the failure of the white blood cell count to increase significantly with infection in our patients with cystic fibrosis as compared to most normal control patients with infection.

An important objection to this study is that the significance of the NBT test has been assessed by clinical judgment together with other available laboratory data. The clinical parameters which usually indicate bacterial infection can be misleading in patients with cystic fibrosis as discussed earlier. At times, the only symptoms of an acute flareup of respiratory disease might be an increased cough. It is also frequently impossible to know if an increase in symptomatology in these patients harboring respiratory pathogens indeed have a bacterial
or viral infection as a cause on many occasions. For this reason, our patients thought to be clinically infected with a probable bacterial infection had this diagnosis made primarily on the judgment of the senior clinician in the cystic fibrosis clinic. Secondary supporting evidence for the diagnosis was made in most cases by the response of the "probable" bacterial infections to antibiotic therapy or to a change in antibiotic therapy, which resulted in a prompt decrease in respiratory symptomatology. There is also abundant evidence, based mainly on studies by Park et al., Feigin et al., and Matula and Paterson, and from our laboratory that a high NBT test correlates very well with the presence of bacterial infection and there is abundant evidence that patients with cystic fibrosis develop recurrent infections in the lower respiratory tract.

I would conclude, based upon the data presented, that the NBT test is a valuable parameter, along with a careful history, physical examination, and other laboratory data in the surveillance and management of patients with cystic fibrosis.
Summary

One hundred fifty seven nitroblue tetrazolium (NBT) dye tests were performed on seventy-eight patients with cystic fibrosis. A "positive" NBT response of $\geq 10\%$ was significantly correlated with the presence of presumed bacterial respiratory infection and stable patients had "normal" NBT scores below 10%. Serial determinations in sixteen instances of bacterial infection were helpful in evaluating response to therapy. Total leukocyte count, mean percentage of immature polymorphonuclear leukocytes, temperature or a change in sputum flora were not helpful in detecting bacterial infection in these patients.

The NBT test is a valuable adjunct in the surveillance of patients with cystic fibrosis.
<table>
<thead>
<tr>
<th>Group I</th>
<th>Shwachman chest x-ray score &gt; 19 stable, doing well.</th>
<th>Age Range</th>
<th>Number with NBT ≤ 10%</th>
<th>Number with NBT &gt; 10%</th>
<th>$\chi^2$</th>
<th>% of neutrophils NBT positive</th>
<th>Absolute number of NBT neutrophils per cu. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-19 1/2 years</td>
<td>38</td>
<td>4*</td>
<td>p&lt; .001</td>
<td>7.4 ± 5.1</td>
<td>(1-32)</td>
<td>444.5 ± 359.5 (14-2061)</td>
</tr>
</tbody>
</table>

| Group II         | Shwachman chest x-ray score > 19 Flare up with increase in respiratory symptomatology and pulmonic process thought to be bacterial | 7 mos. - 18 years | 1        | 18       | p< .001 | 23.1 ± 11.3 (9-56) | 1400.6 ± 1594.6 (335-7285) |

| Group III        | Shwachman chest x-ray score < 19 stable, doing well, on or off antibiotics | 1 - 18 years | 40       | 2        | p< .001 | 6.3 ± 2.5 (2-14) | 453.7 ± 302.3 (79-1520) |

| Group IV         | Shwachman chest x-ray score < 19 Flare up with increase in respiratory symptomatology and pulmonic process thought to be bacterial | 3-26 1/2 years | 2        | 19       | p< .001 | 17.4 ± 5.6 (4-27) | 1423.9 ± 908.7 (134-3562) |

| Group V          | Shwachman chest x-ray score < 19 chronically infected, gradual downhill course with persistent rales and copious sputum production, on continuous antibiotic therapy without amelioration of symptoms | 5-19 1/2 years | 5        | 2        | N.S.    | 9.4 ± 3.4 (5-15) | 945.6 ± 378.6 (580-1598) |

*Two values (11% and 32%) were on one patient taken on two separate occasions. Another patient (20%) developed a fever that night with increased cough but was thought to be stable at the time of physical examination.
Table 2

Correlation of the clinical assessment with NBT test results
in all patients regardless of Shwachman score.

<table>
<thead>
<tr>
<th>Infected by P.E., x-ray and/or history (Groups II &amp; IV)</th>
<th>NBT ≤ 10%</th>
<th>NBT &gt; 10%</th>
<th>Chi - square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>37</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>No active infection by P.E., x-ray or history (Groups I &amp; III)</td>
<td>78</td>
<td>6</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Chronically infected (Group V)</td>
<td>5</td>
<td>2</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Table 3
Correlations of the clinical assessment with various clinical parameters.

<table>
<thead>
<tr>
<th></th>
<th>Clinically not infected</th>
<th>Clinically Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups I &amp; III</td>
<td>Groups II &amp; IV</td>
</tr>
<tr>
<td></td>
<td>84 tests on 62 patients</td>
<td>40 tests on 34 patients</td>
</tr>
<tr>
<td>Mean NBT%</td>
<td>6.8 ± 4.0</td>
<td>20.1 ± 9.1</td>
</tr>
<tr>
<td>Mean Absolute Number of NBT (⁺) Polys per cu. mm.</td>
<td>448 ± 330</td>
<td>1412 ± 1263</td>
</tr>
<tr>
<td>Mean WBC (x 10³)</td>
<td>11.5 ± 3.4</td>
<td>11.5 ± 4.4</td>
</tr>
<tr>
<td>Mean percent &quot;band&quot; forms</td>
<td>2.5 ± 3.6</td>
<td>4.9 ± 6.7</td>
</tr>
<tr>
<td>Mean Temperature (°C)</td>
<td>37.1 ± 0.3</td>
<td>37.7 ± 0.6</td>
</tr>
<tr>
<td>Percentage of patients with a change in sputum flora.</td>
<td>44%</td>
<td>41%</td>
</tr>
</tbody>
</table>

\( \chi^2 \)
Table 4

Correlations of various clinical parameters with the five clinical groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patient</td>
<td>33</td>
<td>18</td>
<td>29</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Number of NBT Tests</td>
<td>42</td>
<td>19</td>
<td>42</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Mean NBT%</td>
<td>7.4 ± 5.1</td>
<td>23.1 ± 11.3</td>
<td>6.3 ± 2.5</td>
<td>17.4 ± 5.6</td>
<td>9.4 ± 3.4</td>
</tr>
<tr>
<td>Mean Absolute Number of (±) polys/cu.mm</td>
<td>444.5 ± 359.5</td>
<td>1400.6 ± 1594.6</td>
<td>453.7 ± 302.3</td>
<td>1423.9 ± 908.7</td>
<td>945.6 ± 376.6</td>
</tr>
<tr>
<td>Mean WBC (x 10³)</td>
<td>11.0 ± 3.2</td>
<td>10.9 ± 5.6</td>
<td>12.1 ± 3.5</td>
<td>12.1 ± 3.1</td>
<td>14.2 ± 2.4</td>
</tr>
<tr>
<td>Mean percent &quot;band&quot; forms</td>
<td>1.9 ± 3.1</td>
<td>4.6 ± 7.1</td>
<td>3.1 ± 3.9</td>
<td>5.2 ± 6.5</td>
<td>10.3 ± 10.8</td>
</tr>
<tr>
<td>Mean Temperature (°F)</td>
<td>98.9 ± 0.6</td>
<td>100.2 ± 1.6</td>
<td>98.8 ± 0.9</td>
<td>99.4 ± 1.6</td>
<td>98.7 ± 0.7</td>
</tr>
</tbody>
</table>
Table 5

Sputum cultures in the five clinical groups

NF = normal flora
P = pseudomonas
S = staphylococcus
C = coliforms
E = enterococci

<table>
<thead>
<tr>
<th>Group</th>
<th>NF</th>
<th>P</th>
<th>S</th>
<th>C</th>
<th>Combination</th>
<th>H. flu</th>
<th>Number of cultures in which there has been a change in the organism(s) isolated from previous cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>21</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>E + S</td>
<td>0</td>
<td>20/42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + β strep</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>α strep + E</td>
<td>0</td>
<td>8/19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>19</td>
<td>1</td>
<td>5</td>
<td>C + E</td>
<td>1</td>
<td>17/42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + P</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C + S + P</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>C + P</td>
<td>0</td>
<td>9/21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S + P</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P + S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ E + H. flu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1/7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>New infiltrate present</td>
<td>No new infiltrate present</td>
<td>Chest x-ray not obtained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>------------------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0</td>
<td>10</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>4</td>
<td>1</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>4</td>
<td>11</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7

Correlation of the presence or absence of rales with the clinical diagnosis and the Shwachman chest x-ray score.

<table>
<thead>
<tr>
<th>Rales Present</th>
<th>Clinically Well (Groups I &amp; III)</th>
<th>Clinically Sick (Groups II &amp; IV)</th>
<th>Chronically Infected (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>9</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Absent</td>
<td>56</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rales Present</th>
<th>Chest x-ray score $\geq 19$</th>
<th>Chest x-ray score $&lt;19$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Absent</td>
<td>43</td>
<td>29</td>
</tr>
<tr>
<td>Grading</td>
<td>Points</td>
<td>General Activity</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Excellent</td>
<td>25</td>
<td>Full normal activity; plays ball, goes to school regularly, etc.</td>
</tr>
<tr>
<td>(36-100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>20</td>
<td>Jacks endurance and tire at end of day; good school attendance</td>
</tr>
<tr>
<td>(71-85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>15</td>
<td>May rest voluntarily during the day; tires easily after exertion; fair school attendance</td>
</tr>
<tr>
<td>(55-70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
<td>Pneum teacher; dyspneic after short walk; rests a great deal</td>
</tr>
<tr>
<td>(41-55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>5</td>
<td>Orthopneic, confined to bed or chair</td>
</tr>
<tr>
<td>(40 or below)</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
Serial NBT determinations in two patients during episodes of respiratory infection.
Figure 2 Scatter Plot of NBT values.
Bibliography


91) Rous, P.: "Relative reaction within living mammalian tissues. II. On mobilization of acid material within cells, and reaction as influenced by cell state." J. Exper. Med. 41: 399, 1925


Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

____________________________________________________________________________________

NAME AND ADDRESS DATE