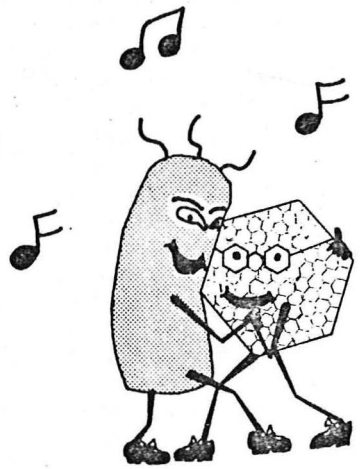
MICROBIAL HARMONIZATION IN HUMAN INFECTIONS



MEDICAL GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL

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INTRODUCTION

The concept of a single microbe producing a single disease forms the basis of our current understanding of the etiology of infectious diseases. This concept originated with the revered "Postulates" of Robert Koch and since at least the time of Erhlich has been a stimulus to clinicians to develop drugs with greater and greater specificity of action against individual microbes. In keeping with this concept, simultaneous infections of patients by more than one microbe have frequently been regarded as little more than fortuitous collections of independent infections - each requiring its own specific mode of therapy.

Gradually, data accumulated by investigators working in a variety of fields are making it increasingly apparent that this concept of the monoetiology of infectious diseases may not always apply to human infections. High degrees of synergism may exist between pathogens in some mixed infections so that the process is more appropriately viewed in terms of a complex of interacting microorganisms than as a collection of independent subsets of infection. Obviously knowledge of such associations in human infections might be of tremendous practical as well as theoretical value. Since no comprehensive review of the subject has previously been published and since this is an area of research that holds tremendous potential for increasing our understanding and ultimate control of a wide variety of human diseases, it is fitting to attempt to summarize the current body of knowledge relating to synergism in human infections.

Of the many different kinds of synergistic activity that have been proposed to exist among human pathogens, all can be codified into one of 4 basic categories:

1. Impairment of host resistance by one microorganism permitting invasion by another microorganism.
2. Increased dissemination of one microorganism as a result of the activity of another microorganism.
3. Provision of elements by one microorganism that are essential to the growth of another microorganism.
4. Increased virulence of one microorganism as a result of factors obtained from another microorganism.

In this review, I will examine those mixed infections in which reasonable evidence for direct synergism between microorganisms has been accumulated and will attempt to bring some order to the diverse and in many cases fragmentary data relating to the mechanisms underlying these associations.

IMPAIRMENT OF HOST RESISTANCE BY ONE MICROORGANISM PERMITTING INVASION BY ANOTHER MICROORGANISM

Case Report:

T.D. is a 47 year old retired marine sergeant who was admitted to the Dallas Veterans Administration Hospital (DVAH) on February 12, 1976 with a two month history of gradually increasing dyspnea on exertion, orthopnea, pedal edema, non-productive cough and a 15 pound weight loss. The patient had spent approximately 10 years in Asia (including 2 tours in Viet Nam) prior to his discharge from the service, but had never been seriously ill nor had he any previous history of cardiac difficulty.

Physical examination revealed the patient to be a well developed, well nourished black male who was in no acute distress. There was jugular venous distention 12 cm above the sternal angle with the patient's thorax elevated at 45°, a summation gallop, dullness at the right lung base with crepitant rales at the left lung base, and 2+ pretibial edema. Chest x-ray showed cardiomegaly with a right pleural effusion. Hematocrit was 31.4%, hemoglobin 10.1 gm, and white blood cell count (WBC) 8,500/mm^3 with a slight shift to the left. Blood chemistries were normal except for a creatinine of 1.5 mg%, blood urea nitrogen 27 mg%, serum glutamic oxalacetic transaminase (SGOT) 95 mU/ml and bilirubin 1.2 mg%. Urinalysis revealed a trace of protein and 3 to 5 WBC per high power field. Pleural fluid obtained from the right hemithorax contained 1.28 gm% protein and 3,113 WBC (86% mononuclear, 14% polymorphonuclear).

Digoxin and diuretic therapy were initiated with the patient experiencing some improvement in his symptoms. However, on February 16 the patient's temperature spiked to 102°F and a new pulmonary infiltrate was noted in the area of the right middle lobe. Numerous blood cultures obtained at this time were sterile (See Table 1), and by February 19 the patient's temperature had returned to normal and his right middle lobe infiltrate appeared to be resolving. On February 22 the patient again became febrile. On the next day he developed sudden tachycardia, hypotension and disorientation. Over the ensuing week, generalized muscle tenderness, a falling hematocrit and precipitously rising SGOT and bilirubin heralded the onset of hemolysis. rhabdomyolysis, and associated acute renal failure. On February 24, *Pseudomonas pseudomallei* was first isolated from the patient's blood. His subsequent hospital course was prolonged and stormy but while receiving a

panoply of antibiotics (including a 6 week course of high dose gentamicin, carbenicillin and doxycycline) the patient gradually improved, and on May 15, 1976 seemed to be cured of his disease and was discharged. Pertinent microbiological studies obtained during his hospitalization are included in the accompanying table:

Table 1

MICROBIOLOGICAL STUDIES OBTAINED DURING FIRST HOSPITALIZATION OF T.D.

Date	Cultures*			Influenza A CF titers
	Blood	Sputum	Urine	
2/18	---			
2/23	--			
2/24	++		+	1:128
2/26	+	+	+	
3/2	++		+	1:64
3/11	++		+	
3/19	+			
3/24	++			
3/31	+++		-	1:32

* -, negative culture; +, *Ps. pseudomallei* isolated from culture

On May 23, 1976, the patient experienced a sudden rigor followed by an episode of syncope. He was readmitted to the DVAH with renewed *Pseudomonas pseudomallei* sepsis and bilateral tibial osteomyelitis. He received another course of combined antibiotic therapy (Carbenicillin, gentamicin and doxycycline) and slowly recovered. He was discharged July 1, 1976 and has been well on a continuous regimen of Septra (Rx) 2 tablets twice a day and doxycycline 100 mg twice a day.

Comment: Initial manifestations of melioidosis appearing at a considerable interval after departure from an endemic area has been described in a number of patients with this disease and in many of these cases, clinical illness seemed to be precipitated by an additional stress to the patient (1). In our particular case, a bout of acute influenza (as evidenced by his clinical picture and falling influenza antibody titer during convalescence) appeared to be the additional stress precipitating acute melioidosis, in some way limiting the host's tenuous control over his latent bacterial infection, so that these bacteria were able to enter a more aggressive phase of invasion.

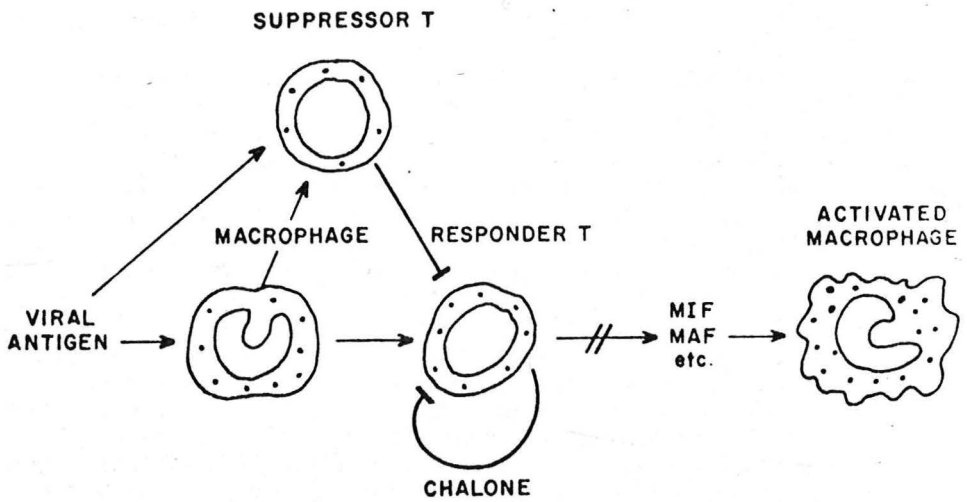
There are a number of ways in which influenza viruses and other microbes may limit man's capacity to fend off secondary invaders. Examples of the specific mechanisms responsible for impairment of host resistance by these pathogens are discussed below:

Depression of Cell-Mediated Immunity:

Depressed cell-mediated immunity has been reported in a wide variety of human infections, including: influenza (2,3), measles (4,5), infectious mononucleosis (6), tuberculosis (5), leprosy (7), syphilis (8), pertussis (9), scarlet fever (10), streptococcal infections (11), disseminated mycotic infections (12), as well as, toxoplasmosis, schistosomiasis and brucellosis (11). In addition to these naturally acquired infections, rubeola, rubella, mumps and attenuated influenza vaccines have also been reported to impair cell-mediated immunity as evidenced by a temporary loss of tuberculin (13) or histoplasmin (14) reactivity.

The precise mechanisms responsible for the impaired cell-mediated immunity in these infections are not known. Notkins and his colleagues (15) have proposed the following hypothesis to account for altered cell-mediated immunity that may accompany viral infections: 1) a direct effect of the virus on the thymus or thymic dependent lymphocytes, 2) virus induced changes on the surface of macrophages and lymphocytes which limit their ability to interact with antigens, and 3) induction or fixation of cytophilic antibody leading to altered cellular immunity. Britton (16) has demonstrated a temporary defect in monocyte migration in infectious mononucleosis and proposes that ablated delayed-hypersensitivity skin reactions occur in this viral infection because of *in vivo* blockade of receptors on monocytes for chemotoxins. Others (17) have found a temporary, though marked, reduction of T-cell rosettes in volunteers with influenza infection. Since incubation of lymphocytes obtained from these patients with thymosin significantly increased the percent of T-cell rosettes, they have suggested that influenzavirus may interfere with the process of differentiation of the T-cells, or that T-cell membranes are altered by the virus in such a way that rosetting does not take place. It has been proposed that anergy may develop in other infections (e.g. miliary tuberculosis) because of extreme antigen excess resulting in binding of all available lymphocyte receptors and in this way preventing these cells from interacting with intradermal antigen (18). Heiss and Palmer (19), after demonstrating an association between anergy and leukocytosis, have suggested that leukocytosis might be the common denominator of anergy induced by infectious agents. Finally, Kantor (11) in an ambitious attempt to construct a mechanistic explanation for infection - induced anergy has proposed a scheme of virus-induced, feedback suppression by lymphocyte products (Figure 1).

Figure 1 (Ref. 11)



Suppression of Responder T Cells by Either Suppressor T Cells or Chalones. T represents T cell, MIF migration inhibition factor, and MAF macrophage aggregation factor.

According to his scheme, viruses might inhibit cell-mediated immunity by stimulating suppressor T-cells that block activation of the responder cell through a mediator substance. Alternatively, responder cells themselves might be stimulated by certain viruses to produce substances (or chalones) which block their activation. This latter pathway has received some support from Kantor's demonstration that such serum inhibitors can be identified in patients with secondary syphilis.

Whatever the mechanism responsible for alterations in cell-mediated immunity that may accompany some infections, relatively little data are available to determine what risk, if any, there is to patients with these infections of secondary invasion by organisms that are normally destroyed by the cell-mediated immune system. Christensen and co-workers (20) in a concentrated, though uncontrolled, study of simultaneous outbreaks of measles and tuberculosis in an isolated community observed depressed skin reactivity and what they felt was an inordinately high incidence of primary as well as reactivation tuberculosis during the measles epidemic. Volkert and others have shown in an experimental model that the course of pulmonary tuberculosis is accelerated during concurrent infections with pneumotropic viruses (21). Although these observations suggest that measles and perhaps other viral infections might increase susceptibility to active tuberculosis, Flick (22), after an extensive review of the literature, has concluded that there are not yet sufficient data to

prove or refute the hypothesis that measles activates tuberculosis. Thus, additional research will have to be undertaken to define the role of pneumotropic viruses in the epidemiology of tuberculosis.

The "stress effects" of vaccination against smallpox and typhoid fever have been shown in one survey (23) to be sufficient to cause a significant increase in the incidence of adenovirus infection. Since recovery from many viral infections is cell-mediated (24), and since smallpox and other viruses have been reported to impair cell-mediated immunity (13,14), this might have been the factor leading to increased susceptibility to adenovirus infections in this survey. Similarly, the well recognized association of herpes labialis and various pyogenic infections (25) might exist because of anergy related to the extreme leukocytosis that accompanies these infections (19). The increased resistance of rabbits to Herpes simplex virus 2 infection after stimulation of their macrophage system with BCG (26) and the dramatic decrease in the recurrence of this infection in patients similarly treated with BCG (27) suggests further that infections that stimulate cell-mediated immunity, in contradistinction to those that suppress it, may increase the resistance of the host to some viral infections.

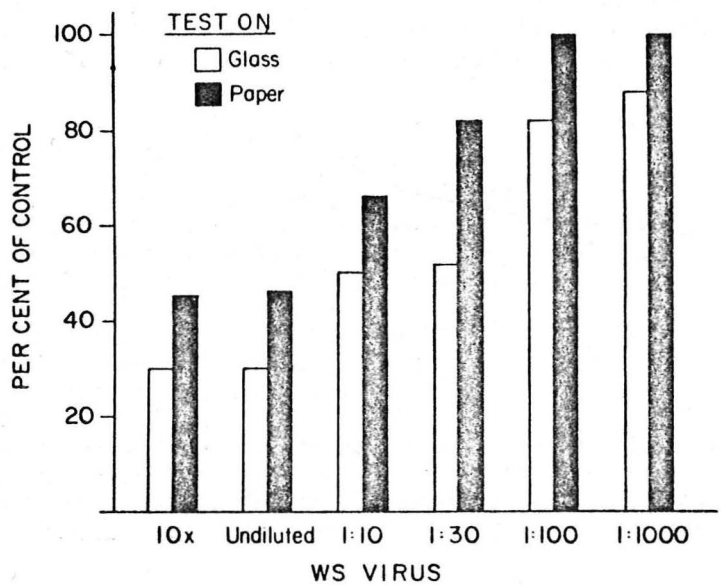
Inhibition of Phagocytosis and the Reticuloendothelial System (R.E.S.):

Although many microorganisms are successful as pathogens in higher animals because they avoid phagocytosis and destruction by the reticuloendothelial system, certain viruses (and perhaps other microorganisms) actually have the capacity to non-specifically impair this important defense system. In this manner, they may provide for the successful invasion of the host by secondary pathogens. Numerous *in vitro* (28-30) and *in vivo* (31,32) experiments have documented the capacity of influenza viruses to inhibit phagocytosis. Other viruses that have been shown to suppress phagocytic activity include: parainfluenza virus (33,34), adenovirus (35), sandfly fever virus (36,37), mumps virus (29), and reovirus (38). Simultaneous infections of laboratory animals with many of these viruses and a variety of bacterial pathogens are associated with a higher mortality rate than infections with either the viral or bacterial pathogens alone (33,38,39), and this increased mortality rate is probably directly related to the inhibitory effect of the viruses on the R.E.S.

Suppression of phagocytic function by viruses is a transient phenomenon (31), and, in at least one system, has been shown to be directly related to the quantity of virus (Figure 2) present and the duration of interaction between the phagocytic cell and virus (32). Viruses do not necessarily have to produce tissue damage in the organ system involved (38) nor do they even have to multiply in the laboratory animal being studied (39) in order to increase the susceptibility of the animal to bacterial superinfection. Although precise mechanisms of inhibition of phagocytosis by viruses have not been defined, preliminary studies indicate that phagocytosis, which is a carbohydrate-dependent function, might falter because the virus induces a metabolic block in the glycolytic pathway of the phagocytic cells (30,37).

Whether this occurs as a result of a direct effect of the virus on the phagocyte, or indirectly through the production of some mediator substance has not been determined.

Figure 2 (Ref. 32)



Relation between the quantity of virus and the inhibition of phagocytosis by guinea pig exudate PMN. Undiluted virus contained $\cdot 10^{8.5}$ EID_{50}/ml. Virus was diluted with NAF. Control cells were incubated with NAF.

The clinical significance of these findings - which represent data accrued for the most part from animal models - remains to be determined. Human studies have provided conflicting data as to the effect of viruses on the phagocytic system of man (35,40), and a great deal more data will have to be obtained before we can answer the question of whether alteration of the phagocytic function of man is the factor responsible for the bacterial superinfections that accompany some human viral infections. Of those clinical situations in which this phenomenon is most likely involved, the propensity of patients with influenza infections for developing secondary bacterial pneumonia with *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus hemolytius* and *Neisseria meningitidis* (41-43) is the most obvious. Patients with measles and adenovirus infections

may be predisposed to bacterial pneumonia for similar reasons (44). It has even been suggested that the non-specific upper respiratory complaints that frequently precede bacterial pneumonia might in some cases represent reovirus infections that predisposed these patients to bacterial pneumonia by decreasing pulmonary clearance (38).

Plasmodia sp., *Bartonella sp.*, and other microorganisms which produce a chronic hemolytic state, may also impair phagocytosis. Unlike the viruses however, they appear to do so in an indirect manner - blockade of the RES due to iron overload (45-47). The consequences of this phenomenon as well as alternative hypotheses as to how these infections increase susceptibility to secondary invaders will be dealt with in a later section.

Alteration of Humoral Immunity:

Various microorganisms have been reported to affect the humoral immune system of man and laboratory animals in such a way as to predispose these hosts to diseases caused by other pathogens. They appear to do so through 3 different pathways:

1. Inhibition of antibody production.
2. Production of sensitizing antibodies.
3. Production of blocking antibodies.

Inhibition of antibody production has been described in association with a wide variety of infections (15). Of the viruses that have been shown to depress humoral immunity, relatively few are agents that infect man and virtually all of the data accumulated have come from studies employing animal models. Of those viruses known to infect man, influenza virus (48), lymphocytic choriomeningitis virus (49), Junin virus (50), and Newcastle disease virus (51) have been shown to depress antibody production in response to heterologous antigen in an animal model.

Several hypotheses have been proposed to explain the depressed humoral immunity that accompanies these viral infections. These hypotheses have recently been summarized by Notkins and colleagues (15) and include: infection of the B lymphocytes by the virus resulting in death or dysfunction of these cells; competitive inhibition of noncommitted antibody-producing cells by the virus; release of endogenous adrenocortical hormones during the course of the virus infections; neoplastic transformation of antibody-precursor cells by the viruses; and (in the newborn) interference with immunological maturation as a result of virus-induced destruction of the thymus.

Viruses may also block antibody production in an indirect manner that has attained clinical relevance only in recent years. It is now known that if a live vaccine such as attenuated poliovirus vaccine is given by the natural route of infection (orally), pre-existing enteroviral infections of the gastrointestinal tract of the vaccinated individual may interfere with the establishment of the vaccine strain (52). Thus, protective antibodies which would normally be expected to follow vaccination

do not develop. In some underdeveloped countries where enteroviruses occupy the intestinal tract of a large percentage of the children much of the time, such interference may pose a real threat to the success of poliovirus immunization programs.

In certain experimental models, endotoxin may temporarily increase susceptibility to various bacterial infections (53). Although the mechanisms responsible for this increased susceptibility are not entirely known, this may in part be due to inhibition of antibody production by the endotoxin (54). Whether this effect of endotoxin contributes to the pathology of mixed infections in man is unknown.

The Plasmodia and perhaps other protozoa may also impair humoral immunity (55). McGregor and Barr (56) have shown that children with malaria are less likely to produce antibodies in response to tetanus antitoxin than counterparts who have been kept free from malaria from birth by continuous chemoprophylaxis. Salaman and co-workers (57) noted a similar diminution in humoral activity in mice infected with malaria. Further evidence of derangement of the humoral immune system during malaria infections has been proposed by Greenwood (58) who observed that recognizable autoimmune disease is less common in African blacks than American blacks in spite of the fact that Africans possess high levels of IgG and IgM, in addition to frequent rheumatoid factor and heterophil antibodies. He suggests that Africans might be relatively insensitive to autoimmune disease because of massive antigenic stimulation of their B lymphocytes by multiple heavy parasitic infestations. This theory has received some support from animal studies showing that infection with *P. berghei* at one month of age protects New Zealand mice from lethal, spontaneous, autoimmune disease (59).

Although malaria and other protozoal infections have been associated with a variety of secondary infections, other factors are probably more important in predisposing the host to these secondary invaders than the effect of malaria on humoral immunity (see later section). Nonetheless, there is increasing evidence that malaria may enhance the oncogenic potential of certain viruses and may, in fact, play a significant role in the epidemiology of at least one human neoplasm - Burkitt's lymphoma. In 1970, Wedderburn (60) showed that the Maloney leukomogenic virus is much more likely to produce lymphoma in mice if these animals are simultaneously infected with *P. berghei* (see Table 2). The Epstein-Bar (EB) virus may be involved in a similar interaction with malaria in man. This is a virus whose main target is the B lymphocyte (61) and a likely cause of Burkitt's lymphoma in man (62). Burkitt (62) has proposed that alteration of lymphoid tissue by malaria may be a factor in the pathogenesis of this malignancy (Figure 3). This hypothesis is consistent with the similar geographic distribution of the 2 diseases and the fact that as discussed earlier, both malaria and the EB virus, affect B lymphocytes.

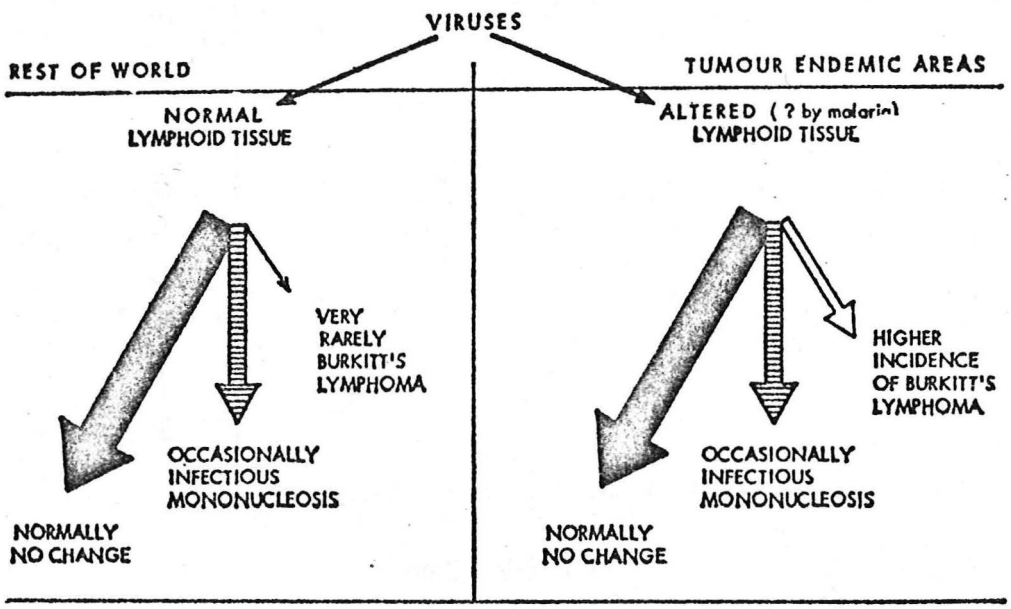
Table 2 (Ref. 58)

INFLUENCE OF FOUR REGIMENS ON INCIDENCE OF LYMPHOMA

Infective agents	No. of lymphomas/ no. inoculated	Latent period (wk.)*
M.L.V.	1/11	20
Pby	0/10	–
Pby + M.L.V. within 5 min.	10/12	15–24
Pby; M.L.V. 10 days later ..	6/13	15½–26†

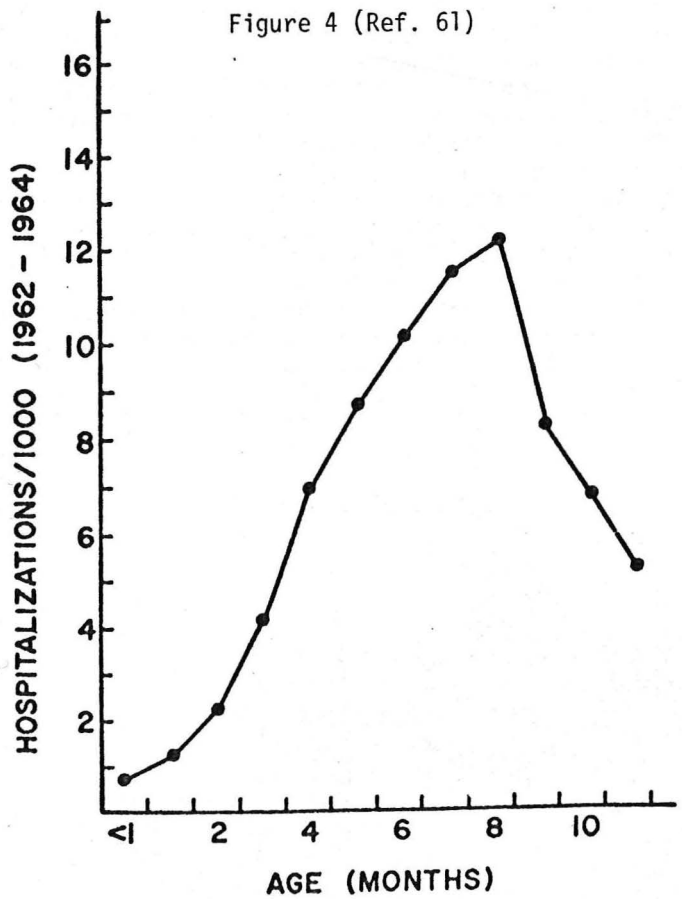
* Survivors killed at 26 weeks. † Measured from injection of M.L.V.

Figure 3 (Ref. 60)



Halstead has recently theorized that one microbe may alter the host's response to a second microorganism by affecting the humoral immune system in a somewhat different manner from that described above (63). According to his theory, Dengue, a relatively mild febrile illness under normal circumstances, may be transformed into a much more severe form of hemorrhagic fever when individuals have circulating heterologous Dengue antibodies at the time of their infection (usually with Dengue virus type 2). Halstead proposes that prior infection with a heterologous strain of Dengue virus or passive transfer of antibodies from mother to infant, results in circulating IgG antibodies which "sensitize" the individual to a second infection. He points out that the occurrence of this syndrome during the first year of life (Figure 4) suggests that antibody and not sensitized cells mediate Dengue virus hypersensitivity.

Figure 4 (Ref. 61)



Average annual age-specific DHF hospitalization rates for infants under 1 yr old

Since IgG, which is the primary antibody crossing the placenta, has never been shown to mediate hypersensitivity reactions, he feels that Dengue hemorrhagic fever may represent antigen-antibody complex disease where the antibody is pre-existing IgG.

Finally, studies recently reported by Griffiss and Bertram (64) suggest that certain microorganisms may increase susceptibility to secondary invaders by stimulating production of IgA-blocking antibodies that shield the secondary pathogens from circulating IgM. In a study of serum obtained from 28 patients with meningococcal disease, these investigators showed that 24 of their patients possessed sera that was deficient in meningococcal-bactericidal activity. Surprisingly, when they removed serum IgA by absorption with an anti-IgA: Sepharose immunoabsorbent, bactericidal activity was uniformly present at a dilution of 1:100. Thus, susceptibility to disseminated meningococcal disease seems to correlate with absence of specific, serum bactericidal activity (normally present in 80-90% of young adults) and circulating IgA appears to be responsible for this deficiency by blocking bactericidal IgM.

Griffiss has proposed the following hypothesis to explain the origin of IgA blocking antibodies (65): Enteric bacteria with surface mucopolysaccharides similar to meningococci colonize the individual and through their interaction with the antibody-forming cells of Peyer's patches and other intestinal lymphocytes, initiate the production of secretory IgA antibodies. Activated cells migrate from Peyer's patches (Figure 5) to the mucus membrane of the upper respiratory tract through the interrelated network of local immunity that has been recognized as common to all mucosal surfaces (66-69). In their new location, the IgA-producing cells may then produce IgA which coats a later arriving meningococcus with an appropriate mucopolysaccharide capsule and in so doing unwittingly protects this organism from the bactericidal effects of circulating IgM. Although highly speculative at this point, Griffiss feels that this hypothesis might account for sudden epidemics of meningococcal disease in selected populations such as the armed services and deserves additional investigative consideration.

Figure 5 (Ref. 67)

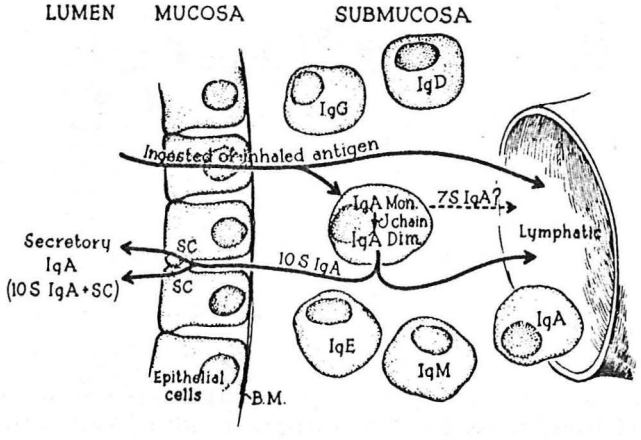
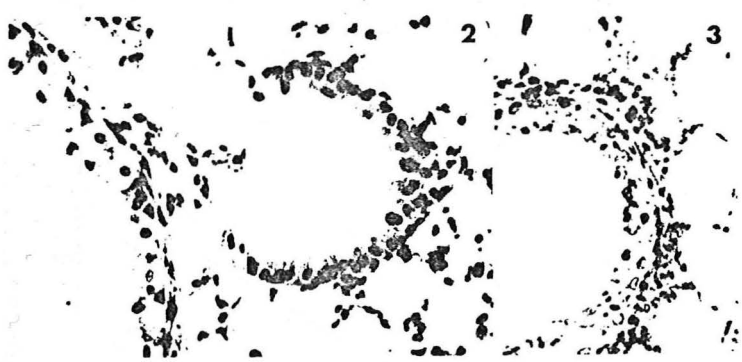


Diagram Showing the Sites of Synthesis and Routes of Transport of Immunoglobulins from the Submucosal Area of a Secretory Organ such as the Gastrointestinal Tract.

Anatomical Alterations:

In early attempts to identify specific factors responsible for the increased susceptibility of patients with influenza infections to pyogenic pulmonary infections, Harford and his co-workers (70) examined the pulmonary clearance of inhaled pneumococci in mice with influenza infections. They showed that normal mice and mice with early influenza infections cleared inhaled pneumococci with equal rapidity, however, mice with late influenza infections had impairment of pulmonary clearance to such a degree that pneumococci actually increased in number following introduction into the lung. Necropsy examination of the lungs of these animals showed early osmotic changes in the bronchial epithelium (Halo cells) of control animals that had received intrabronchial diluent alone and in infected animals that had received influenza virus and diluent (Plate 1; Figure 6). Six hours after intrabronchial challenge, bronchial epithelial cells of all animals appeared normal (Plate 2; Figure 6), however, 48 hours after intrabronchial inoculation of a lethal dose of influenza virus there was diffuse necrosis of bronchial epithelium in infected animals (Plate 3; Figure 6).

Figure 6 (Ref. 70)



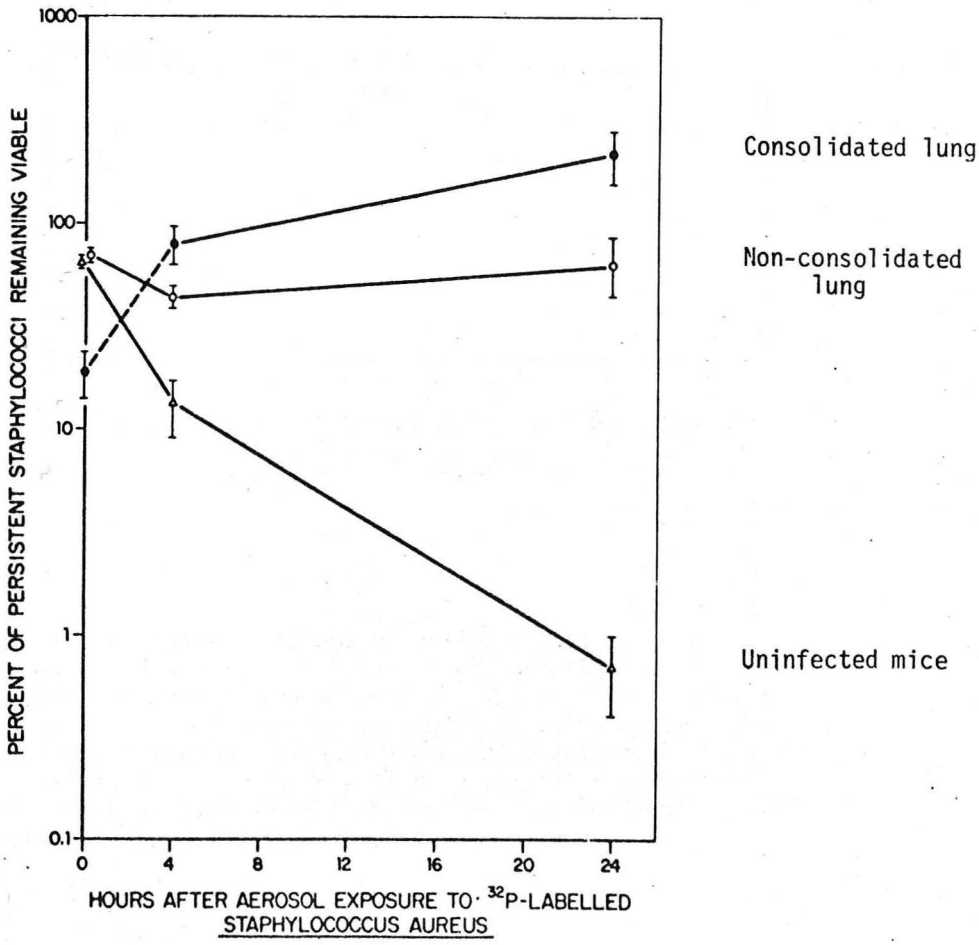
Bronchial Epithelial Changes in
Experimental Influenza

Since only those animals with extensive necrosis of bronchial epithelium exhibited a reduced capacity to clear inhaled pneumococci, the authors concluded that these histologic changes, in some ill-defined way, promoted growth of pneumococci.

These same investigators also showed that artificially induced pulmonary edema similarly impaired pulmonary clearance of pneumococci in their mouse model (71), and that bronchial ciliary activity persists in mice even with severe influenza infections (72). They proposed that the edema and cellular debris that accompany influenza infections provide a suitable culture media for secondary bacterial pathogens and is the prime factor responsible for the association between influenza and pyogenic pneumonia.

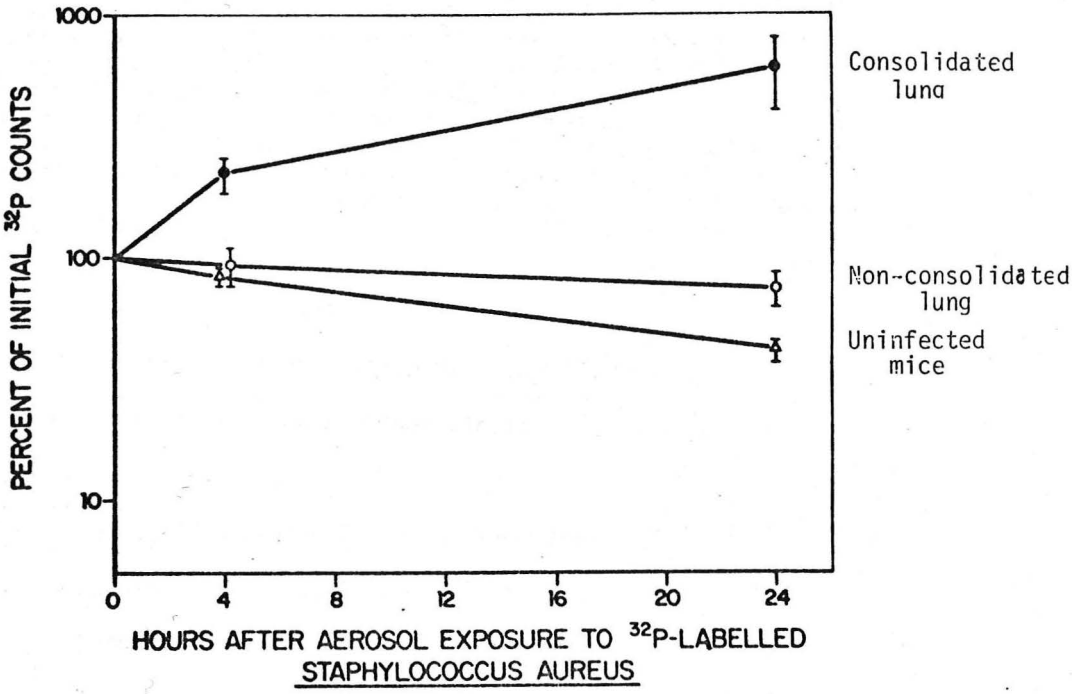
More recent work by Jakab and Green (34) suggests that the association between viral and bacterial pulmonary infections might be somewhat more complex. In their studies they examined the rate of clearance of inhaled staphylococci from consolidated and non-consolidated areas of lung in mice that had previously been infected with Sendai virus. Bactericidal activity was depressed in both consolidated and non-consolidated lung (Figure 7).

Figure 7 (Ref. 34)



Nonetheless, bacterial multiplication occurred only in consolidated areas of lung (Figure 8) and was associated with a reduction of the physical transport system in this area. The authors hypothesized that the apparent multiplication of bacteria in consolidated areas of Sendai virus pneumonia may have reflected either transport of organisms toward these areas or spread of consolidation into previously non-consolidated areas.

Figure 8 (Ref. 34)



It has been estimated that 50% of patients with bacterial pneumonias have evidence of underlying viral infections (73). In all likelihood, the many varied effects of viruses on the resistance of the host that have been reviewed, each contributes to this close association between them and secondary bacterial pathogens. Which effects are the most important in a given situation - such as in our patient with acute melioidosis precipitated by influenza - can only be speculated upon until additional data become available.

The association between pulmonary aspergilloma and tuberculosis is well recognized (74,75) and represents a classic example of how one organism, through anatomical alterations of the host, may predispose to infection by another. Saprophytic colonization by *Aspergillus sp.* of cavities formed by another infectious agent has also been reported to occur in bronchiectasis (76), lung abscesses (75,76), and various pulmonary mycoses (74,77,78). Although sporadic reports of concomitant tuberculosis and mycotic infections other than aspergillosis (79-81) suggest that the tuberculous cavity might provide a suitable environment for other fungi, tuberculosis appears to have been the secondary (rather than predisposing) infection in many of these cases and may, in fact, have developed because of exposure of the patients to *M. tuberculosis* during the work up of their mycotic infection at T.B. sanatoriums (80).

In a classic study published in 1942, Baum and Amberson (82) demonstrated yet another way in which anatomical changes produced by one organism might affect the course of infection by another. In a retrospective review of the records of 102 episodes of pneumonia in 97 tuberculous patients admitted to Bellevue Hospital between 1930 and 1941, they identified 8 "definite" and 10 "probable" instances in which activation of latent tuberculosis or an increase in the severity of chronic active tuberculosis developed as a consequence of pyogenic pneumonia (Table 3).

Table 3 (Ref. 82)

PNEUMONIA ASSOCIATED WITH TUBERCULOSIS

Definite Activation of Tuberculosis	8
Due to Pneumonia	4
Due to Suppuration following Pneumonia	4
Probable Activation of Tuberculosis	10
Due to Pneumonia	9
Due to Suppuration following Pneumonia	1
Doubtful Activation of Tuberculosis	11
No Activation of Tuberculosis	65
Not Known	8

The pneumococcus was the most common pyogenic organism isolated from these patients (Table 4). Activation of the tuberculous infection was most likely to occur if the pyogenic infection occupied the same lobe as the tuberculous focus, leading the authors to conclude that "activation of tuberculosis by a suppurative disease is in the majority of cases dependent on local destructive processes, resulting in disruption of the capsule, if any, and breaking down of the tuberculous focus".

Table 4 (Ref. 82)

TYPES OF PNEUMONIA ASSOCIATION WITH TUBERCULOSIS

TYPE OF ORGANISM	NUMBER OF CASES	DEFINITE ACTIVATION OF TUBERCULOSIS	PROBABLE ACTIVATION OF TUBERCULOSIS	BACTERAEMIA	DEATH
PNEUMOCOCCI	48	6	7	7	10
MIXED	7	-	2	-	-
UNCLASSIFIED	7	-	1	1	2
STREPTOCOCCUS HAEMOLYTICUS	4	2	-	1	1
MIXED OTHER THAN PNEUMONIA	7	-	-	-	-
UNKNOWN	29	-	-	-	4
TOTAL	102	8	10	9	17

Haltalin and Nelson (83) recently proposed a similar mechanism for the association between shigellosis and bacteremia due to other gram negative bacilli. They reported 3 cases of Aerobacter septicemia complicating shigellosis in children and concluded that mucosal ulcerations produced by *Shigella sp.* were responsible for the bacteremia by providing access of the secondary invaders to the blood stream.

Studies conducted in laboratory animals suggest that viruses that infect renal cells may predispose the host to bacterial pyelonephritis because of anatomical changes in the kidneys which accompany these infections. By examining the effect of renal infections of adult mice with mouse adenovirus, herpes simplex virus and vaccinia virus on susceptibility to pyelonephritis after challenge with intravenous or retrograde *E. coli*, Ginder (84) has been able to demonstrate a significant predisposition to bacterial pyelonephritis in mice that have antecedent viral urinary tract infections. He has suggested that tubular obstruction that accompanies viral-induced tissue necrosis in the kidneys of these animals is responsible for the increased susceptibility to bacterial pyelonephritis. Although numerous viral infections of man produce viruria and presumably involve the urinary tract (84-87), no obvious association between viral infections and bacterial pyelonephritis has been documented in man.

Schistosoma haematobium has been reported to predispose man to secondary invasion of his urinary tract by Salmonellae, and apparently does so because of its capacity to produce anatomical alterations of the human urinary tract that favor survival of the salmonellae (88,89). In a study of salmonella infections in Egyptians, Hathout and others (89) found 21 chronic, urinary salmonella carriers among 54 patients who had been simultaneously infected with schistosomiasis at the time of their acute salmonella infection. In contrast, none of the 21 patients without concomittant schistosomiasis became chronic urinary carriers. Radiological studies of carriers revealed a high incidence of obstructive uropathy - presumably due to schistosomiasis.

Neisseria gonorrheae probably plays a similar role in the pathogenesis of acute pelvic inflammatory disease. It does so by producing adhesions in the Fallopian tubes which pave the way for secondary invaders from the vagina. Thus, while, the gonococcus is rarely isolated from cultures of the cul-de-sac of patients with acute salpingitis (137), it probably plays a prominent role in the pathogenesis of this infection and does so by producing anatomical changes of the host.

The ability of group A streptococci to produce structural changes of man's cardiac valves through the induction of acute rheumatic fever is well known (90). Mechanisms of tissue injury induced by group A streptococci (91) as well as the consequences of these valvular lesions as a predisposing factor to infection by secondary pathogens (92) have already been extensively reviewed and therefore will not be addressed further at this time. Nonetheless, subacute bacterial endocarditis developing on a valve previously damaged by an attack of acute rheumatic fever is one of the most graphic illustrations of one organism paving the way for invasion by another as a result of anatomical alterations of the host.

INCREASED DISSEMINATION OF ONE MICROORGANISM AS A RESULT OF THE ACTIVITY OF ANOTHER MICROORGANISM

Case Report:

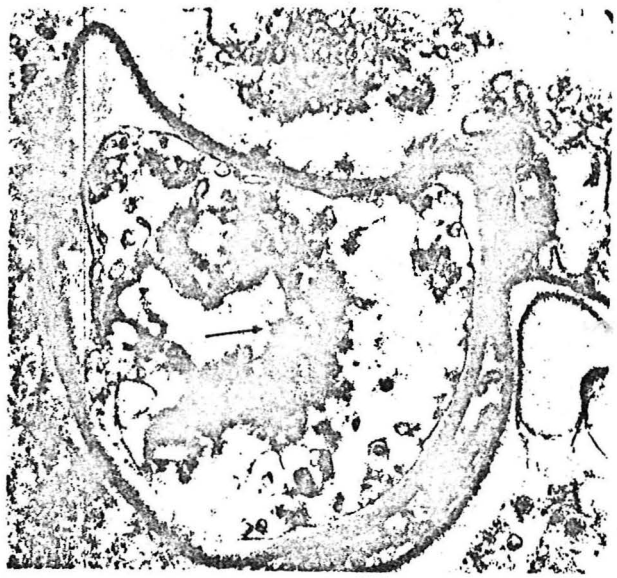
E.T. was a 51 year old businessman with end-stage chronic glomerulonephritis who was admitted to Parkland Memorial Hospital on June 2, 1972 for elective renal transplantation. He had had known renal disease for 28 years and at the time of this admission complained of easy fatigability, burning of his fingers and toes and generalized pruritus. Physical examination revealed a pale, elderly-looking, white man who was in no acute distress. The patient was afebrile with a blood pressure of 140/100 mm Hg. There was early hypertensive retinopathy, a grade I/IV systolic murmur at the apex and decreased position sense in the fifth toes bilaterally. Laboratory studies revealed: Blood urea nitrogen: 122 mg%; serum creatinine: 11.5 mg%; 4+ proteinuria.

On July 5, the patient received a related living donor renal transplant. He experienced a stormy post-operative course with increasing azotemia in spite of therapy with azathioprine, 200 mg per day and prednisone, 100 mg per day. On July 21, the patient's condition began to deteriorate rapidly with fever, marked tachypnea, disorientation and a diffuse interstitial pulmonary infiltrate. Herpes labialis was also noted at this time. Bronchoscopy was performed and was unremarkable except for a few acid fast bacilli identified in the bronchial washings that were obtained. Gomori methenamine silver stains of the washings showed neither *Pneumocystis carinii* or fungi. Broad-spectrum therapy that included gentamicin, carbenicillin, methicillin, isoniazid, ethambutol and pentamidine was initiated to no avail and the patient expired on July 28, 1972. An autopsy was performed and revealed chronic glomerulonephritis, chronic rejection of the renal transplant, cytomegalic inclusion virus pneumonia, *Pneumocystis carinii* pneumonia and herpes labialis. Cultures of the bronchial washings in which acid-fast organisms had been seen were sterile.

Comment: This patient illustrates a common problem of the immunosuppressed patient - opportunistic infection. Not only is the immunosuppressed patient likely to become infected, but frequently he is simultaneously infected with multiple pathogens. Our patient was simultaneously infected with cytomegalovirus, *Pneumocystis carinii* and Herpes simplex virus, but apparently not, as initially suspected, with *M. tuberculosis*.

Although it is certainly not uncommon to have infection with more than one organism in immunosuppressed patients (93), the combination of *Pneumocystis carinii* and cytomegalovirus (CMV) infections, occurs much more frequently in these patients than one would expect to see by chance alone (94,95). Of the many hypotheses that have been proposed to explain this association, one recently proposed by Wang, Huang and Thurlbeck (95) is particularly intriguing. These authors observed CMV-like bodies within *P. carinii* in two patients with combined Pneumocystis and CMV pneumonia (Figure 9).

Figure 9 (Ref. 95)



Electron Micrograph of *Pneumocystis carinii* Showing Small Round CMV-like body (arrow).

They could not be certain that the spherical bodies they observed were not normal organelles of the Pneumocystis, but thought this unlikely in view of the fact that these structures have not been a consistent finding in electron micrographs of *P. carinii* published by other groups. Consequently they proposed that the parasitized Pneumocystis might act as a vector for CMV or, alternatively, that the patient and *P. carinii* are infected simultaneously in

these mixed infections. Obviously, this hypothesis must be regarded as tentative until it can be tested by more sophisticated techniques to determine if the structures within the Pneumocystis are in fact CMV. Alternative hypotheses such as activation of a dormant form of *Pneumocystis carinii* by CMV should be pursued at the same time.

There are, nonetheless, a number of situations in which fairly good evidence has been accumulated to show that one microorganism may act as a vector for another. The most famous of these was originally described by Shope in 1941 (96), in which the Swine lungworm acts as the reservoir and intermediate host for swine influenza virus. Shope was intrigued by agrarian folklore which held that earthworms were, in some unknown fashion, responsible for the persistence of hog cholera virus from one outbreak of the disease to the next. He therefore set out to determine if the swine lungworm (*Metastrongylus sp.*; Figure 10), a nematode parasite that resides in the bronchioles of swine and has the earthworm as an intermediate host, might act as a vector between the hog and this mythical earthworm reservoir.

Figure 10 (Ref. 96)

Third-stage lungworm larvae as seen in a fresh "press" preparation of the calciferous gland of an experimentally infested earthworm. × 94. Photographed by Mr. Julian A. Carlile.

Although Shope was unsuccessful in his initial attempts to demonstrate an earthworm cycle for the hog cholera virus, he had luckily chosen the swine influenza virus as his control virus. Much to his surprise, this virus was able to persist in a masked form within the swine lungworm for long periods, so that years might elapse between its transmission from one swine to the next. Shope showed that the virus normally exists within its vector in a non-infective form, and a provocative stimulus (such as injections of *Hemophilus influenzae species* or the migration of ascaris larvae) is necessary to initiate overt influenza infection in the hog. Experimentation by other groups have further substantiated Shope's hypothesis of the epidemiology of swine influenza (97,98). In later studies, Shope (99) found that the swine lungworm can also serve as reservoir and intermediate host for the hog cholera virus and that provocation of latent infections in swine may occur with migration of ascaris larvae through their lungs. More recently, Shotts and co-workers (98) have developed a laboratory model in which *Strongyloides ratti* (an intestinal threadworm) serves as a vector for swine influenza virus in murine populations. Thus a variety of viruses, nematode vectors and hosts appear to have the capacity to participate in a cycle similar to that originally described by Shope.

Although there is no evidence of similar forms of masked infection in man, Sanford (100) points out that there are epidemiologic features of human influenza that are not adequately explained by the classic concept of man-to-man transmission. He sites as an example the 1789 and 1918 influenza outbreaks in which spread of influenza appeared to travel more rapidly than available transportation would have permitted. Whether or not nematode reservoirs might have played a role in these outbreaks, or participate in the epidemiology of other human viral infections remains to be seen.

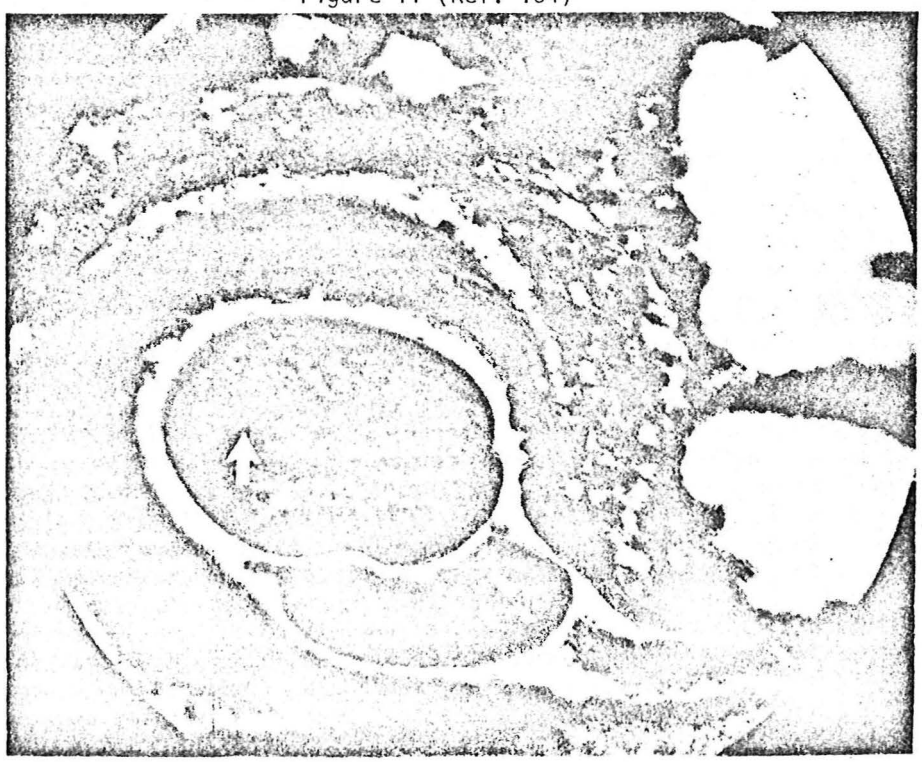
Numerous investigators have reported a peculiar association between schistosomiasis and Salmonella infections in man (101-103). Although the mechanisms underlying this association remain largely unknown, there is evidence that Schistosomes themselves become colonized by bacteria and as such might serve as a reservoir for Salmonella in man (104,105).

In areas of the world where *Schistosoma mansoni* is common, clinicians have observed an unusual form of salmonellosis characterized by a prolonged febrile course, weight loss, marked hepatosplenomegaly and chronic Salmonella bacteremia (102,103). Surprisingly, patients tolerate these long intervals of infection well, suggesting that the usual relationship between Salmonella and man is altered from that seen in other kinds of Salmonella infection (103). The fact that the majority of these patients are simultaneously infected with *S. mansoni* (101-103) suggests that this organism may play a role in producing this altered relationship.

Schistosoma may alter the mammalian host in a variety of ways. Mice, for instance, exhibit little change in resistance to *S. typhimurium* when simultaneously infected with *Schistosoma mansoni*, whereas early Schistosoma infections produce increased resistance to infection by *Listeria monocytogenes* and decreased resistance late in the course of Schistosomiasis (106). Collins and colleagues (106) have suggested that early resistance is seen because of "stimulation" of the RE system of these animals by Schistosoma antigens whereas the reduced resistance seen in later infections eventuates because of fibrosis of the liver and spleen and associated atrophy of the R.E.S. In man hepatosplenic schistosomiasis has been reported to be associated with decreased lymphocyte migration (107) and reduced serum-anti-Salmonella activity (108). These studies provide a variety of potential mechanisms to explain the altered resistance to Salmonella infection that may occur in patients with schistosomiasis.

A number of experimental observations suggest an important alternative explanation for how Schistosoma might serve as a chronic focus for low grade salmonella bacteremia. In 1940 Krakower (104) observed that in experimental *S. mansoni* infections in rats, many worms became spontaneously infected with gram positive bacilli late in the course of the schistosomal infection. These bacilli localized in the cecum of the worm (Figure 11) where they frequently multiplied rapidly and destroyed the infected worm.

Figure 11 (Ref. 104)



Schistosoma in Branch of Pulmonary Artery of the Rat with Early Bacterial Invasion of the Cecum (arrow).

These interesting observations have subsequently been expanded upon by Ottens and Dickerson (105) who were able to cure mice of *S. mansoni* infections by inoculating them with Klebsiella and other gram negative bacilli which parasitized and destroyed Schistosomes in 3-4 days. Their observation that gram positive bacteria did not have a similar effect on the Schistosomes suggests that different bacteria may interact with these worms in different ways. In light of these data, it has been suggested that colonization of the ceca of Schistosomes by Salmonella might provide a reservoir for Salmonella in patients with the chronic form of Salmonellosis described above (103). It has also been speculated that at some future date man might have the capacity to cure himself of *S. mansoni* infection by using an appropriate bacterium to destroy this parasite (109).

Another example of metazoa influencing non-metazoan infections of mammals has recently been reported by Cypess and collaborators (110). In a laboratory model using *Nematospiroides dubius* (a nematode parasite of the upper intestine of mice), streptomycin-resistant enteropathogenic *E. coli* and streptomycin-treated mice, they observed heavier *E. coli* infestations of mice that were simultaneously parasitized by *N. dubius*. Since *E. coli* counts were highest in the area of the bowel occupied by *N. dubius* and since motility of parasitized bowel is reduced (111), they concluded that increased bacterial colonization of the small bowel occurred in *N. dubius* infected mice because of changes in the "motility, integrity and consistency of the parasitized gut". Alternative hypotheses such as colonization of *N. dubius* itself by *E. coli* as a mechanism of increased colonization of parasitized bowel were not addressed. Nonetheless, their study raises the, as yet untested, hypothesis that certain parasites might participate in the epidemiology of bacterial gastroenteritis in man.

Dr. Heinz F. Eichenwald and his co-workers (112-114) have provided us with a clear concept of another way in which one microorganism may facilitate transmission of another from host to host. In studies of colonization of the newborn infant's nasopharynx by potentially pathogenic bacteria, they demonstrated a correlation between viral upper respiratory infections and colonization by Staphylococci, streptococci, pneumococci and *Haemophilus influenzae* (Table 5). Some of the infants with simultaneous isolation of a respiratory virus and bacterial pathogen from their nasopharynges exhibited a "stuffy-nose syndrome" characterized by frequent sneezing, snorting and coughing. This syndrome was distinctly less common among those infants who had respiratory virus or bacterial pathogen alone isolated from the nasopharynx (Table 6). Because of these symptoms, infants with "stuffy-nose syndrome" constantly contaminated their contiguous physical environment and air space with virus and bacteria. A small number of infants without symptoms also actively disseminated bacteria from their nasopharynx. These, like those with the "stuffy-nose syndrome" were generally concomitantly infected with a respiratory virus and bacterial pathogen. Because infants of this group were literally surrounded by clouds of bacteria, they were referred to as "cloud babies" by the authors The relationship between simultaneous adenovirus and staphylococcus infection and aerial dissemination of staphylococci in one such infant is illustrated in Figure 12.

TABLE 5 (Ref. 114)

COLONIZATION OF NASOPHARYNX BY VARIOUS BACTERIA AMONG NEWBORN INFANTS WITH OR WITHOUT SIMULTANEOUS VIRAL INFECTIONS OF UPPER RESPIRATORY TRACT

	Total No. of Colonized Infants	Virus Present*	Virus Absent†
β-Hemolytic streptococcus	18	16	2
Haemophilus influenzae	14	13	1
Pneumococcus	10	10	0
Staphylococcus aureus, type 52A/79	23	20	3
S. aureus, type 47/77/83	14	12	2

* Isolated and passed in tissue culture at least once during or just preceding period of bacterial colonization.
† No cytopathogenic agent recovered on at least three occasions.

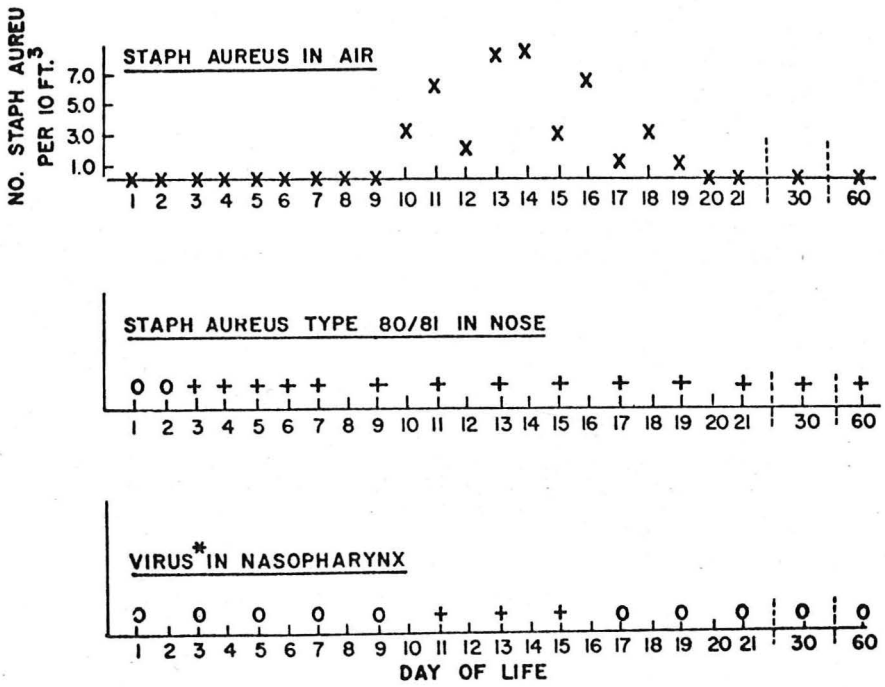
TABLE 6 (Ref. 114)

RELATIONSHIP OF STAPHYLOCOCCUS AUREUS AND ADENOVIRUS TYPE 1 TO AN OUTBREAK OF STUFFY-NOSE SYNDROME IN PREMATURE INFANTS

Infectious Agents Isolated from Nasopharynx	Total No. Infants	No. with Stuffy Nose	Per cent of Group with Stuffy Nose
Adenovirus 1 and *S. aureus**	30	30	100
Adenovirus 1 and miscellaneous bacteria	22	0	0
*S. aureus**, no virus	41	2	5

**S. aureus*, coagulase positive, hemolysin positive, phage type 29/47/7/77.

Figure 12 (Ref. 114)



Relationship of simultaneous virus and staphylococcus infection to aerial dissemination of staphylococci (infant L.L., 4,050 g). * Unidentified agent cytopathogenic to rhesus kidney and human amnion.

Eichenwald and his colleagues have proposed that the "stuffy-nose syndrome" might represent one of a number of possible phenomena: simple additative irritation of the nasopharynx by the two pathogens; modification of one microbe by mucus produced in response to the other microbe; or alteration of the local defenses of the host by one microbe in such a way as to enable more florid multiplication of the other. Alternatively, they proposed that "cloud babies" might disseminate organisms in the absence of symptoms because: the virus increased the number of bacteria present in the oropharynx without producing symptoms in the host; increased production of a mucus vehicle occurred and was unnoticed in infants with dual infections; or the viscosity of the mucus might have been altered in such a way as to promote dispersion. Whatever the reasons, it is clear from these studies that the normal capacity of the newborn infant's nasopharynx to reject a surprising variety of pathogenic bacteria (115,116) is dramatically reduced during active viral infections of

the upper respiratory tract. Furthermore, virus and pathogenic bacteria appear to act in concert in their effect on the host to promote their dissemination to other potential hosts.

Similar findings in studies of older individuals (117-121) and experimental animals (122,123) suggest that this kind of microbial synergism is not limited to newborn infants but may be widespread among man and other mammals. Gwaltney and his co-workers (121) recently showed an association between intrafamilial spread of *S. pneumoniae* and rhinovirus infections. In a simultaneous outbreak of meningococcal and influenza infections, investigators from the Center for Disease Control (124) demonstrated a similar correlation between the meningococcal carrier state and influenza infection (Table 7).

TABLE 7 (Ref. 124)

Relation between Meningococcal Carrier State and Serologic Evidence of Influenza.*

Serologic Evidence of Influenza	Meningococcal Carriers		% Meningococcal Carriers
	YES	NO	
Positive	4	20	17
Negative	0	24	0 †p = 0.054
Totals	4	44	

*Excluding carriers who later had influenza.

Other investigators have also reported simultaneous meningococcal infections or bacterial meningitis and viral infections in numerous case reports (125-127) and small outbreaks (128,129). Although these reports suggest that there are an unlimited number of bacterial-viral combinations that may act in concert to predispose man to bacterial meningitis, the relatively rare occurrence of these mixed infections (126) and a report by Eickoff (130) of a lack of correlation between serological evidence of meningococcal infection and adenovirus infections do not support such a conclusion. Whether Eichenwald's "cloud baby" hypothesis is an adequate explanation for the occurrence of these simultaneous infections, or they involve one of the other modes of synergism discussed in this paper, or they are simply fortuitous associations will require additional research efforts to determine.

PROVISION OF ELEMENTS BY ONE MICROORGANISM THAT ARE ESSENTIAL TO THE GROWTH OF ANOTHER MICROORGANISM

Case Report:

W.H. was an 84 year old retired retailer who came to the Dallas Veterans Administration Hospital on May 18, 1975 complaining of difficulty opening his mouth and of having punctured his foot with a nail approximately one week prior to admission. Physical examination revealed a partially drained abscess of his right foot. This was incised and drained and the patient was given an injection of tetanus toxoid. A short time after this procedure the patient experienced a grand mal seizure and arrested. He was promptly resuscitated but because of repeated grand mal seizures had to be paralyzed with pancuronium bromide and placed on a mechanical ventilator. His subsequent hospital course was characterized by extreme irritability with frequent tetanic contractions and increasing respiratory insufficiency secondary to congestive heart failure and bronchopneumonia. In spite of vigorous supportive efforts he expired on May 25. Permission for autopsy was denied. Aerobic cultures of pus obtained from the abscess of the right foot yielded *Serratia marcescens*; anaerobic cultures were not performed.

Comment: This man's clinical presentation of tetanus leaves no doubt that *Clostridia tetani* was present in his foot abscess and producing toxin in spite of the fact that appropriate efforts had not been undertaken to isolate the organism.

According to Gorbach and Bartlett (131) infections associated with anaerobic organisms are almost always mixed and lend themselves better to models of bacterial synergy than almost any known microbial systems. Even tetanus, which our patient was unfortunate enough to acquire, may involve a complex system of synergistic activity. It has been shown that tetanus spores will not germinate if inoculated alone into experimental animals, however, if concurrently introduced with facultative bacteria, proper oxidation-reduction potentials ensue, enabling tetanus spores to germinate, grow and produce toxin (131,132). It is likely that the *Serratia marcescens* obtained from the abscess of our patient played a pivotal role in providing a suitable environment for germination and growth of tetanus spores in his abscess. A similar function may be performed by the guinea worm (*Dracuncula medinensis*) to produce the high incidence of tetanus that accompanies dracunculiasis in certain tropical countries (133).

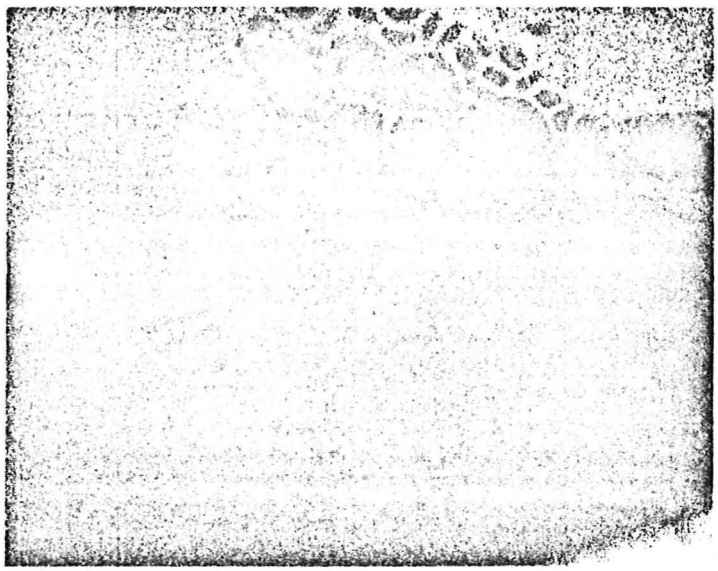
Synergistic gangrene of Meleney (134) illustrates another example of how facultative microorganisms may provide a more favorable environment for anaerobes. This is a progressive infection of the skin involving a microaerophilic, nonhemolytic streptococcus and a hemolytic *Staphylococcus aureus*. When the two organisms are inoculated together (but not individually) into laboratory animals the characteristic disease develops - presumably because the staphylococcus permits the streptococcus to assume invasive properties by producing hyaluronidase and a heat labile "growth factor" (135).

Necrotic anaerobic processes involving mucous membranes appear to involve a somewhat different mode of synergism (131,126). *B. melanogenicus* is most likely the major pathogen in these infections, but because of an obligatory requirement for vitamin K (136) does not flourish unless this metabolic requirement is satisfied. Normally, this essential element is provided by a facultative diphtheroid.

These are but a few examples of how facultative bacteria and obligate anaerobes may act in concert to produce human infections. They, and other as yet unidentified mechanisms, almost certainly play a prominent role in the mixed infections of the abdomen, chest and surgical wounds. They are important to recognize not only because it is intellectually satisfying to understand the pathophysiology of human diseases, but also because knowledge of these crucial microbial alliances has, in certain instances, enabled the clinician to develop more rational therapeutic programs. A prime example, is the observation that patients with mixed anaerobic infections of the lung may respond well to limited antibiotic therapy directed at a segment of the total microbial population and not require regimens tailored to the individual destruction of each member of the infectious process (131,137). Patients with penicillin-resistant *Bacteroides fragilis* or gram negative bacilli present in their (mixed) anaerobic pulmonary infections appear to respond quite well to penicillin as the single antimicrobial agent (137), and this response probably reflects a symbiotic dependency of the penicillin-resistant microorganisms on their penicillin-sensitive accomplices. Thus, by approaching the infection as a single intensely unified entity rather than a fortuitous association of independent microbes, the clinician has been able to develop a more rational therapeutic approach to the disease.

Spontaneous infections involving *Toxoplasma gondii* and CMV occur with surprising frequency among patients with disseminated cancer (138-140) and may represent a symbiotic association comparable to those that have been described for mixed anaerobic infections. Gelderman and his co-workers (141) showed that these two organisms may occupy the same cells in patients with dual infections. Furthermore, the growth pattern within these cells is peculiar in that the *T. gondii* rosettes tend to form a ring around the CMV inclusion body (Figure 13). These investigators have proposed that mitochondria which are concentrated around the CMV inclusion body may serve as an energy source for *T. gondii*.

Figure 13 (Ref. 141)



Comparison of toxoplasma replicating in a non-virus infected cell (top) and a virus infected one (below).

Malaria and other hemolytic infections may assist certain secondary invaders through a similar process of providing the secondary pathogen with an essential nutrient (142). In experiments that provide some insight into the mechanisms responsible for the well recognized association (142) between malaria, bartonellosis, viral hepatitis and other iron overload states and infections with such secondary invaders as Salmonella and Listeria, Masawe and Nsanzumuhire (143) performed *in vitro* studies of bacterial growth in blood from patients with iron deficiency and hemolytic anemias. They showed that blood from patients with hemolytic anemia supported growth of test organisms better than controls while blood from patients with iron-deficiency anemia grew fewer organisms than controls. In subsequent studies (47), their group showed that patients with iron-deficiency anemia had significantly fewer bacterial infections than non-iron deficient subjects living in the same tropical community. Others have shown that injections of ferric ammonium citrate greatly increase the mortality rate of mice infected with *Yersinia enterocolitica* and the septicemic form of this disease may be more common among humans with iron overload (144).

The findings of these different groups of investigators are probably best understood in the light of a recent proposal (142) that an important mechanism of defense of the mammalian host is his capacity to withhold iron from pathogenic microorganisms by sequestering it away in complexes with transferrin. Accordingly, patients with hemolytic anemia or hepatitis, because of an excess of circulating free iron have a reduced capacity to withhold this essential nutrilite from potential bacterial pathogens. Thus, as proposed by Weinberg and his colleagues (142), the production of an iron-overload state may be one mechanism by which infections like malaria or hepatitis predispose the host to secondary bacterial infections. Impairment of the function of the R.E.S. may also occur in certain hemolytic infections due to erythrophagocytosis (145) and as mentioned in an earlier section this may be a factor contributing to the increased susceptibility of patients with these infections to secondary invaders. What the individual significance of these two different effects or the impairment of humoral immunity that may accompany malaria (55-57) might be with respect to secondary infections will require additional investigative work to determine.

Perhaps the most dramatic of all microbial systems in which one microorganism has been shown to provide elements essential to the growth of another are those well known to the virologists in which a "helper" virus is required by certain "defective" viruses to code for an enzyme(s) essential to their reproduction (146,147). This process has been given the name complementation (148) and is a phenomenon that has been identified as a feature of the life cycle of at least one group of human viruses - the adeno-associated viruses. They have been classified as parvoviruses and are defective in human cells, but will reproduce when cultured in conjunction with adenoviruses with which they cohabit the human throat. Although these viruses illustrate an important virological phenomenon, the defective viruses have not yet been causally related to human diseases.

INCREASED VIRULENCE OF ONE MICROORGANISM AS A RESULT OF FACTORS OBTAINED FROM ANOTHER MICROORGANISM

Case Report:

L.O. was a 5 year old Latin-American female who was admitted to Parkland Memorial Hospital on May 21, 1968 with a 4 day history of lethargy, chills, fever and sore throat. She had had no prior immunizations. Physical exam revealed an acutely ill-appearing child with obvious swelling of her neck and a peculiarly pungent odor to her breath. Temperature was 102°F, pulse 104 per minute, respirations 34 per minute and blood pressure 95/55 mm Hg. A tough, foul-smelling, grey-white membrane covered the tonsils, uvula and posterior pharynx. The remainder of the physical examination was unremarkable.

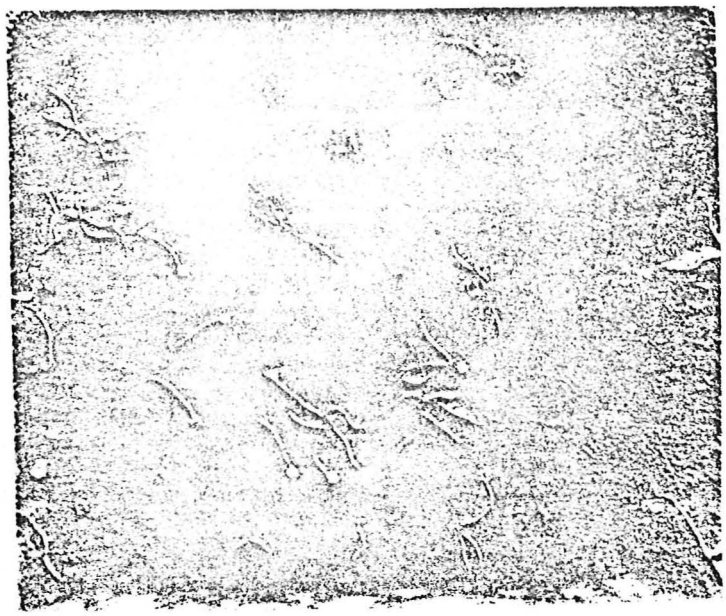
Initial laboratory studies included: hematocrit 40%, hemoglobin 13.5 gm, white blood cell count 18,800 per cu mm and normal blood chemistries. The urine contained 300 mg% protein and 2-6 red blood cells per high-power field. Chest x-ray and electrocardiogram were within normal limits. Schick test was positive and throat culture yielded toxigenic *Corynebacterium diphtheriae*.

The patient was treated with diphtheria antitoxin and intravenous penicillin and appeared to improve slightly during her initial hospitalization. On May 25 partial right bundle branch block developed and the patient's blood urea nitrogen and creatinine began to rise. On May 27, transient 3^{o} heart block appeared followed by alternating right and left bundle branch block. Approximately one month after admission, the patient began to have difficulty swallowing and was noted to have paralysis of her palatal musculature. Because of associated respiratory difficulty, she received a tracheostomy and was placed on a mechanical respirator. Two days later the patient's endotracheal tube became clogged with debris and she arrested. Irreversible hypoxic brain damage developed as a consequence of this arrest and the patient has been unresponsive to verbal communication since that time. She was last seen at PMH in November of 1974 at which time she was noted to have generalized spastic paralysis and cortical blindness and was being cared for by her mother at home.

Comment: This unfortunate child illustrates many of the clinical features of diphtheria and their potential tragic consequences.

Diphtheria is a disease which graphically illustrates the phenomenon of interaction between different microorganisms which results in increased virulence of one of these microorganisms. Through the work of a number of investigators (149-152) we now know that toxin production is a bacteriophage-mediated property of toxigenic strains of *C. diphtheriae* and that avirulent strains of this organism may be converted to virulent strains by infection with the bacteriophage illustrated in Figure 14. The most current evidence suggests that toxin production and bacteriophage synthesis occur simultaneously and that iron is important in the latter stages of release of toxin (153).

Figure 14 (Ref. 152)



Electron micrograph of bacteriophage B.

Lysogeny (or symbiosis of a bacterium with a phage) is also common among group A streptococci and may be another example of increased virulence of a bacterium resulting from infection by a virus (154). There is increasing evidence that production of scarlatinal toxin may be a phage-mediated process among some group A streptococci (153,154). Whether lysogeny contributes significantly to the ability of these organisms to produce the various streptococcal disease states is not yet known, however the association of lysogeny with various streptococcal disease states is not yet known, however the association of lysogeny with various epidemic strains of streptococci has suggested to some investigators (154) that it is an important area for future research in attempts to define the mechanisms responsible for these disease states.

Lysogeny has also been observed among staphylococci and at least three toxins produced by these bacteria appear to be in some manner phage-mediated (153). Acquisition of alpha toxin (155) and enterotoxin "A" (156) production following lysogenization has been demonstrated in experiments with staphylococci Suppression of beta toxin production following lysogenization has been observed in other investigations (157). The clinical significance of these experiments is at present unknown.

Transmission of drug-resistance factors (R factors) is another well recognized example of interactions between microorganisms that result in increased virulence of one of the participants (158-161). As a result of transfer of these limited pieces of genetic material from one cell to another, one bacterium may increase the resistance of another to one or more antibiotic agents. That this phenomenon has clinical relevance is illustrated by numerous outbreaks of drug-resistant bacterial infections in which the offending bacteria appear to have acquired their resistance from R-factors (158,162-164). Recent studies in which segments of R-factors have been successfully transposed to bacteriophages (165,166) suggest that under rare circumstances viruses may participate in this process.

In certain experimental situations, antibiotic degrading enzymes derived from one bacterium may act in a more direct fashion to protect other sensitive bacteria. Tacking (167) has shown that when experimentally induced wound abscesses in rabbits contain bacterial strains that produce large amounts of extracellular penicillins and a penicillin-sensitive bacteria, penicillinase produced by the resistant bacteria will inhibit the effect of penicillin on both the resistant and the sensitive bacteria. Similar results have been obtained by other groups working with different experimental models (168,169). It has not yet been determined whether or not this mechanism is important in mixed infections in man.

Entamoeba histolytica is well known for its ability to produce highly destructive lesions of the large bowel, liver and other organs of man. Nonetheless, it exists primarily as a commensual in the large intestine where it feeds on bacteria and less commonly on superficial mucosal cells (170). Why, under rare circumstances, this organism may become highly invasive is a mystery, however, the work of some investigators suggest that virulence may be mediated by intestinal bacteria. Axenic cultured ameba have been shown to be avirulent when injected into the hamster livers but if allowed to associate with certain bacterial species for 6 to 12 hours will produce liver abscesses in these same animals (171). Neither bacterial filtrates nor killed bacteria possess the virulence factor necessary to effect this transition nor does growth of amebae and bacteria in individual chambers separated by a semipermeable membrane result in increased virulence of the amebae. Similarly, germ-free guinea pigs are refractory to *E. histolytica* infections and will only develop "normal" infections unless *Clostridria perfringens* or *Bacteroides fragilis* are present in the bowel of these animals (170).

SUMMARY AND CONCLUSIONS

In this paper, I have attempted to review the current status of our understanding of microbial synergism as it relates to human disease. It is increasingly apparent that significant degrees of synergism do exist between pathogens in many mixed infections and that the concept of the monoetiology of infectious diseases embodied in Koch's postulates may not always apply to human infections. As seen in this review, even infections as familiar and seemingly uncomplicated as lobar pneumonia, influenza, tetanus, amebiasis and diphtheria may involve latent synergism between two or more microbes.

Although a wide variety of microorganisms have been recognized as participants in this kind of activity, the mechanisms of microbial synergism can all be codified into one of four basic categories:

1. Impairment of host resistance by one microorganism permitting invasion by another microorganism.
2. Increased dissemination of one microorganism as a result of the activity of another microorganism.
3. Provision of elements by one microorganism that are essential to the growth of another microorganism.
4. Increased virulence of one microorganism as a result of factors obtained from another microorganism.

The composite information currently available on this subject are at best rudimentary. Consequently, our concepts of the pathophysiology of most mixed human infections must be regarded as tentative. In fact, many of the hypotheses summarized in this paper will probably have to be discarded as additional experimental data become available. Nonetheless, this is an area of research which holds tremendous promise for increasing our understanding and ultimate control of a wide variety of human diseases and as such deserves our most concerted attention.

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