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The Role of Microbial Interactions in Infectious Disease

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The role of microbial interactions in infectious disease

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The occurrence of infectious disease is affected by interaction between microorganisms in three ways.

The indigenous flora (commensal microorganisms) of some mucous surfaces provide one of the main protective mechanisms against infection by pathogens (disease-producing microbes). The commensal populations interfere with the establishment of pathogens on mucous membranes by evoking anaerobic conditions, by competing for space and nutrients and by producing inhibitors. How, at the beginning of successful infection, pathogens in relatively small numbers overcome this protective activity of the commensal population is unknown.

Although not a general phenomenon, some pathogens exacerbate the effects of others. The best examples are the potentiation of bacterial infections by existing viral infections: mucosal adherence and penetration by bacteria are enhanced and phagocytic defences against them weakened.

Some microorganisms that are unable to produce significant disease on their own may combine with others to cause serious sickness. The harmful effects of these combinations of microorganisms can be explained by the multifactorial nature of pathogenicity (virulence), i.e. the capacity to produce disease. Although each member of the mixed population cannot alone produce the full complement of factors needed for disease production, the complement can be attained by combining contributions from different members.

Introduction

There are three major areas where interactions between microorganisms affect the production of infectious disease. Firstly, the indigenous microbial flora (commensal microorganisms) on some mucous surfaces have a strong protective function against potential invaders. Secondly, some pathogenic microbes can potentiate or exacerbate the effect of others. Thirdly, microbes that are unable to cause significant disease alone can combine with other similar organisms to cause harmful, even fatal, infections. I shall take each area in turn and describe, with examples, the evidence for the occurrence of the phenomena before discussing the possible mechanisms concerned.

The protective effect of commensal microorganisms on mucous surfaces

Some mucous surfaces, such as the upper respiratory tract, the buccal cavity, the lower bowel, the endo-cervix and the vagina, abound with commensal microorganisms. Others, for example the alveoli, the upper intestine, the urethra and uterus, are devoid or relatively free of indigenous flora. When present, commensal microbes appear to have a strong protective effect against infection.

In passing, it should be noted that the skin has a commensal population that protects by processes similar to those about to be described for mucous surfaces (Mims 1976). However,

because of the overwhelming influence of the mechanical strength of the skin, the commensal microbes are much less important in overall protection against infection than for the mucous surfaces and consequently their effects on the skin have been studied less. For these reasons only commensal protection of mucous surfaces is discussed here.

(*a*) *Evidence for a protective effect*

Treatment of normal animals with antibiotics reduces or removes their commensal bacteria and this allows pathogens to infect mucous surfaces more easily. In humans, after treatment with antibiotics for various diseases, secondary infections arise, e.g. oral and vaginal thrush due to *Candida albicans* (Odds 1979), pseudomembranous colitis due to *Clostridium difficile* (Rolfe *et al.* 1981) and nasal infections with *Staphylococcus aureus* (Wannamaker 1980). Experimentally, the treatment of laboratory animals with antibiotics has enhanced subsequent enteric infections with *C. albicans*, *Vibrio cholerae*, shigellae and salmonellae (Savage 1972).

Mucosal infection of gnotobiotic animals with pathogens is reduced by mixing with the whole flora of normal animals or members of it. Examples of such experiments using gnotobiotic mice are; the inhibition of intestinal infection by the C25 strain of *Escherichia coli* and its translocation to the mesenteric lymph gland by the normal flora of SPF mice (Berg & Owens 1979); the inhibition of intestinal infection with shigellae by components of normal human intestinal flora (Maier & Hentges 1972); and the reduction of alimentary tract candidiasis by *E. coli* (Nishikawa *et al.* 1969).

Newly hatched chickens have been protected from infection with food poisoning salmonellas by oral administration of the caecal contents of adult birds or anaerobic cultures of them (Rantala & Nurmi 1973; Barnes *et al.* 1980). The protective caecal material contained lactobacilli, coliforms, faecal streptococci and anaerobes but attempts to protect with lactobacilli alone or mixed with various anaerobes failed (Barnes *et al.* 1980).

Less relevant than the above experiments with animals but offering supporting evidence for a protective effect of commensal microbes are the results of mixed cultures *in vitro* involving pathogens and members of the mucosal flora. For example, the multiplication *in vitro* of *Pseudomonas aeruginosa*, various enterobacteria and *Shigella flexneri* was inhibited by normal intestinal bacteria (Hentges & Maier 1970; Levison 1973). Also, the growth of gonococci *in vitro* was reduced by aerobic and facultatively anaerobic members of human endocervical flora in one study (Saigh *et al.* 1978) and by strict anaerobes in another (Morin *et al.* 1980). *Cl. difficile* was inhibited *in vitro* by strains of six bacterial species present in human gut flora particularly lactobacilli and group D enterococci (Rolfe *et al.* 1981).

(*b*) *The mechanisms of protection*

Production of low oxygen tension, competition for nutrients, limiting adherence by occupation of mucosal space and formation of inhibitors all probably contribute to protection against pathogens, but the evidence is often circumstantial rather than solid.

Usage of oxygen by commensals can produce anaerobic microenvironments not only in sites like the lower bowel but in oxygenated areas such as the teeth and gums (Savage 1977; Jayawardene & Goldner 1975; Loesche 1976). In these anaerobic environments both commensal and pathogenic anaerobes (clostridia, *Bacteroides* spp. and spirochaetes) can flourish, but pathogens requiring oxygen for significant growth would be restricted.

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Metabolic competition for the available nutrients is an obvious method whereby commensal populations could restrict mucosal multiplication of pathogens but, as far as I am aware, only Freter and his colleagues (Freter *et al.* 1973) have investigated the possibility experimentally. First, it was shown that the complete caecal contents of normal mice or a mixture of 100 anaerobic Gram-negative bacteria isolated from these contents (the so called 'F strains') restricted the growth of *E. coli* strain C 25 (and also shigellae) in gnotobiotic mice. Then, continuous-flow cultures of the complete caecal contents or the mixed F strains were established in anaerobic (10 % H_2, 5 % CO_2, 85 % N_2, O_2 less than 5 $\mu l\ l^{-1}$) glove boxes by using a medium of veal infusion broth enriched with yeast extract, haemin and menadione. The compositions of the normal flora and the 'F strains' were preserved for at least 3 months in such cultures. When the 3-month-old cultures were fed to gnotobiotic mice, the intestines of the latter became conventionalized in a similar manner to when fresh caecal contents were used. Thus, the two anaerobic continuous cultures simulated the conditions *in vivo*. When the streptomycin resistant (to allow separate counting) strain C 25 of *E. coli* was included in either of the cultures for 4 weeks, its population levelled off at 10^6–10^7 ml^{-1} compared with 10^8–10^9 ml^{-1} in pure cultures. Also, the filtered effluent from either of the mixed cultures was a poor growth medium for *E. coli* strain C 25 in contrast to the original medium and, unlike in the latter, addition of glucose to the effluent markedly stimulated growth. Clearly, the whole caecal contents and the 'F strains' were restricting growth of the *E. coli* C 25 by competitive utilization of carbon sources in the continuous culture medium. Although this work was not conducted with members of a pathogenic species such as shigellae, it clearly indicates that metabolic competition can restrict growth.

To infect mucous membranes successfully, pathogens must adhere to the surfaces of epithelial cells or the covering mucus; otherwise they would be removed by moving lumen contents (Freter 1980). However, commensal microbes are often closely associated with epithelial surfaces, sometimes in thick multilayered populations (Savage 1972, 1977). Thus pathogens could be excluded physically from mucosal surfaces by commensal microorganisms, and the effects of the latter on host cell receptors, mucosal enzymes and rates of mucosal cell turnover could adversely effect adherence of pathogens (Freter 1980). Whether these potential protective effects of commensal populations operate *in vivo* is unknown.

Similarly, inhibitors of pathogens are produced by commensal microbes but their relevance *in vivo* is often equivocal. Volatile, and short-chain non-volatile fatty acids are formed by anaerobic bacteria in the lower bowel and, in a reducing environment, appear to be weakly bactericidal or growth-inhibiting to pathogens such as shigellae and salmonellae (Meynell & Subbaiah 1963; Savage 1972, 1977; Levison 1973; Bryne & Darkert 1979). The protective effect of adult caecal contents on baby chickens has been attributed to such acids (Barnes *et al.* 1980), but not everyone agrees on their role (Freter *et al.* 1973). Hydrogen sulphide, ammonia and hydrogen peroxide have also been quoted as inhibitors of pathogens in the alimentary tract (Savage 1977; Wannamaker 1980), but their effects have been investigated less than those of lower fatty acids. Lactic acid produced by Doderlein's bacillus and other lactobacilli in the vagina is said to be protective against urogenital infections in women (Mims 1976) and it may be a mediator in the protective effect of chicken caecal contents (Barnes *et al.* 1980). That bacteriocins could have a protective effect *in vivo* was shown by the experiments of Braude & Siemienski (1968): after inoculation of one kidney of a rat with a bacteriocin-positive strain of *E. coli* and the other with a bacteriocin-negative strain, the latter was eliminated by the former in the subsequent bladder infection. Turning to their possible role on mucous surfaces, oral streptococci may prevent

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buccal cavity infection by other bacteria through the production of bacteriocins. They are called viridins and inhibit Gram-negative as well as other Gram-positive organisms (Wannamaker 1980). Formation of antibiotics by some commensal organisms has also been suggested (Savage 1972, 1977) as a protective mechanism, but there is little evidence that it operates *in vivo*. Finally, some recent work (Roach & Tannock 1980) suggests that indigenous micro-organisms may stimulate systemic host defence mechanisms against attack by a pathogen such as *Salmonella typhimurium*.

(*c*) *How are the protective mechanisms overcome by pathogens?*

The simple answer is that we do not know. Occasionally, relatively few organisms of a pathogenic species can overcome the powerful protective mechanisms of commensal micro-organisms. How this is accomplished in areas like the lower bowel is a mystery.

Ducleuzeau & Raibaud (1974) showed that *Shigella flexneri* could mount a resistance mechanism against elimination by *E. coli* in gnotobiotic mice. When *Sh. flexneri* was grown in gnotobiotic mice for 1 day before the introduction of *E. coli* it was eliminated in 8 days. If, on the other hand, the shigellae were present alone in the mice for 3 months, resistance to elimination by *E. coli* was elicited and was complete at the end of the period, although in mixed culture *in vitro*, the shigellae adapted *in vivo* were still eliminated by *E. coli*. The nature of this resistance acquired *in vivo* to commensal elimination, which if it occurred in humans would be transferred by faecal contamination in epidemics of dysentery, is not known. As far as I am aware, these interesting observations on shigellae have not been followed up.

Production of bacteriocins by β-haemolytic streptococci in the buccal cavity, and by group D streptococci in the urinary tract, may give them an advantage over some commensals (Wannamaker 1980). Also, supporting a possible role for bacteriocins, colonization of the teeth of gnotobiotic rats by bacteriocin-positive strains of *Streptococcus mutans* occurred at the expense of bacteriocin-negative strains when both strains formed a mixed inoculum (Rogers *et al.* 1979).

Cooperation between pathogens, especially viruses and bacteria

Infection with one pathogen might not affect the course of infection with another, for example, in mice, tuberculosis and mycoplasmal infection had little influence on superimposed anthrax and staphylococcal infection respectively (Henderson 1964; Howard *et al.* 1978). Sometimes pathogens can be antagonistic to one another; thus guinea pigs with brucellosis were protected against respiratory anthrax (Henderson 1964), and staphylococci appear to inhibit infections with *Pseudomonas aeruginosa* (Shebl & Al-Sawagh 1980). Cooperation between pathogens can also take place and this leads to more severe disease than that produced by them alone. The clinically important examples occur between viruses and bacteria and they are discussed here because the mechanisms concerned have been investigated experimentally.

(*a*) *The enhancement of bacterial infections by viruses*

In natural disease of man, there are strong clinical indications that respiratory viruses commonly increase the susceptibility of the respiratory tract to infection with bacterial pathogens, particularly staphylococci, pneumococci and *Haemophilus influenzae*. In measles, secondary

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bacterial infection with these organisms is most frequently manifested as otitis media, bronchopneumonia and mastoiditis (Tamm & Horsfall 1965) and exacerbation of tuberculosis appears to occur (Fenner & White 1976). The unpleasant effects of the common cold, namely purulent nasal discharge, otitis, sinusitis, catarrh and, in children, fever and lower respiratory infection (Cherry *et al.* 1967), result mainly from bacterial superinfection. Infections with adenoviruses and herpes viruses may also predispose patients to bacterial infections (Green *et al.* 1977; Kleinerman *et al.* 1974). Influenza, however, is the best example; alone it is rarely lethal but secondary bacterial infections following it were a scourge before the advent of antibiotics and even now are often fatal (Stuart-Harris 1965; Mulder & Hers 1972). Haemolytic streptococci used to cause most trouble but have virtually disappeared in favour of streptococci, pneumococci and to a lesser extent *H. influenzae* (Sweet & Smith 1980). In the veterinary field similar exacerbation of bacterial infection by viruses occurs in the respiratory tract (Howard *et al.* 1978) and between rotaviruses and enteropathogenic *E. coli* in the intestine (Dubourguier *et al.* 1978; Gouet *et al.* 1978).

Experimentally, bacterial infections have been superimposed at varying times after inoculation of viruses into laboratory animals. Comparison of the bacterial contents of appropriate tissues with those of animals receiving the bacteria alone demonstrated an enhancement of infection. Exacerbation of disease was shown by more severe lesions or greater death rates than in animals receiving either the bacteria or virus alone. The classic experiment in this mould was that of Shope (1931) using influenza virus and *H. influenzae* in pigs. Since then mice have been the main experimental animals, and examples of many investigations with them are as follows. Influenza virus enhanced respiratory infections with pneumococci (Harford *et al.* 1949), anthrax bacilli (Henderson 1964), staphylococci (Jakab *et al.* 1979) and *Listeria monocytogenes* (Gardner 1980). Sendai virus enhanced respiratory infections with *H. influenzae*, staphylococci, *Pasteurella pneumotropica* and *Mycoplasma pulmonis* (Degree & Glasgow 1968; Howard *et al.* 1978). Adenovirus, reovirus and cytomegalovirus enhanced infections with *E. coli* (Ginder 1964), staphylococci (Klein *et al.* 1969) and *Ps. aeruginosa* (Hamilton & Overall 1978) respectively.

(*b*) *Mechanisms of viral enhancement of bacterial infections*

To cause disease a microorganism must be able to (1) infect a mucous surface, (2) penetrate into the tissues, (3) grow in the tissue environment, (4) interfere with host defence mechanisms, and (5) cause damage to the tissues of the host (Smith 1978). The following experiments show that viruses exert their effect by increasing the ability of bacterial pathogens to achieve one or more of these steps. The majority of investigations have entailed influenza virus or Sendai virus with staphylococci, pneumococci or *H. influenzae*. Respiratory infections of mice have been the main vehicle but there have been some relevant observations on human volunteers.

Bacterial infection of respiratory tract surfaces could occur more easily if mucociliary clearance was impaired by virus attack or if adherence to epithelial cells was enhanced. The first seemed not to occur in mice infected with Sendai virus, since trachiobronchial clearance of inhaled radiotracer-labelled bacteria was not significantly altered (Green *et al.* 1977). Similarly, mucociliary clearance appeared to be unimpaired during influenza of mice despite damage to ciliated epithelium (Harford & Hamlin 1952). On the other hand, bacterial adherence to host cells appears to be enhanced by prior virus infection. Thus, staphylococci adhered more to pharyngeal cells of patients with naturally acquired respiratory illness than to cells from uninfected people (Fainstein *et al.* 1980). Also, staphylococci, pneumococci and *H. influenzae* adhered

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more to the pharyngeal cells of volunteers experimentally infected with influenza virus than to corresponding cells from an uninfected control group (Fainstein *et al.* 1980). In mice, *Ps. aeruginosa* adhered to trachael cells better when the latter had been damaged by infection with influenza virus (Ramphal *et al.* 1980). The increased adherence of staphylococci may have been mediated by interaction of their protein A with antibody against influenza virus attached to antigens on the surfaces of infected cells; thus the adherence of protein A-containing staphylococci to influenza virus-infected cells was enhanced by antibody to influenza virus in contrast to the adherence of strains not possessing protein A (Austin & Daniels 1978). Both pneumococci and streptococci can also contain protein A-like molecules (Austin & Daniels 1978). Another possible mechanism for increased bacterial adherence to virus-infected cells was indicated by the possible mediation of the adherence of group B streptococci to influenza virus-infected cells by the viral haemagglutinin expressed at the cell surface (Sanford *et al.* 1978). However, this type of enhanced adherence has not yet been demonstrated with influenza virus and a bacterial species whose infection is enhanced *in vivo* (Sanford *et al.* 1978).

Penetration of tissues by bacteria must be aided by the denudation of epithelial cells that results from acute infection with influenza virus (Stuart-Harris 1965; Mulder & Hers 1972). For example, staphylococci appear to attack only those parts of the respiratory tract that have been damaged by the virus (Mulder & Hers 1972).

Viral promotion of bacterial growth in the tissues might occur for pneumococci in the lungs of mice infected with influenza virus. Viral infection alone induced lung oedema and virus-free oedematous fluid from such mice enhanced the ability of pneumococci to produce pneumonia in fresh mice not previously infected with influenza virus (Harford & Hara 1950).

Turning to the inhibition by viruses of host defence mechanisms against bacteria, impairment of phagocytes has received most attention. Experiments *in vitro* showed that many viruses (vaccinia virus, influenza virus, Sendai virus, Newcastle disease virus, herpes simplex virus, measles virus) were cytotoxic to polymorphonuclear phagocytes and macrophages; chemotaxis was depressed and the ability to ingest bacteria impaired (Smith 1980). *In vivo* influenza of mice depressed macrophage accumulation at inflammatory sites (Kleinerman *et al.* 1976) and impaired their capacity to remove staphylococci from the lungs (Jakab *et al.* 1979); both ingestion and killing of bacteria by the alveolar macrophages was inhibited (Warshauer *et al.* 1977). Mice infected with reovirus types 2 and 3 exhibited decreased clearance of staphylococci from the lung due to decreased phagocytic activity (Green *et al.* 1977). Inhaled staphylococci were ingested equally by alveolar macrophages of normal mice infected with Sendai virus but the bacteria were killed in the former cells and proliferated in the latter (Green *et al.* 1977). In similar experiments, but with *Candida krusei* as the test organism for probing the efficiency of phagocyte defences, both ingestion and phagosome–lysosome fusion were inhibited in alveolar macrophages of mice infected with Sendai virus (Jakab *et al.* 1980). Despite the overwhelming weight of evidence that influenza, Sendai and other viruses impair phagocytic function, thus allowing bacteria to infect more easily, a few studies with influenza virus (Nugent & Pesanti 1979) and Sendai virus (Mills 1979) have failed to demonstrate the impairment. The reason for this is unknown.

Finally, it is possible that viruses and bacteria combine to damage host tissues more than they do individually, but I am not aware of any proven example.

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Disease produced by mixtures of non-pathogenic or weakly pathogenic microorganisms

Some veterinary and human diseases are associated with a mixed microbial flora, the members of which are non-pathogenic or only weakly pathogenic when acting alone. In some cases the interacting microorganisms have been identified and in others not. Proving Koch's Postulates becomes increasingly difficult with the rise in the number of different microbes possibly involved in one clinical syndrome.

Heel abscess or infective bulbar necrosis in sheep occurs when the hoof and interdigital tissue are exposed to a mixed microbial flora from faeces under wet conditions (Roberts 1969). *Fusiformis necrophorus*, accompanied by *Corynebacterium pyogenes*, penetrates into the dermis and causes severe necrosis. Experimental inoculation of *F. necrophorus* or *C. pyogenes* alone produced little damage, but the typical lesion was reproduced by a mixture (Roberts 1969). Calf respiratory disease is another important veterinary condition due to a mixed aetiology, the responsible components of which have not yet been completely resolved. Two viruses (para-influenza virus type 3 and respiratory syncytial virus), three mycoplasmas (*Mycoplasma dispar*, *Mycoplasma bovis* and *Ureaplasma sp.*) and several bacteria (*Pasteurella multicida*, *Pasteurella haemolytica*, staphylococci, streptococci and *Haemophilus somnus*) in various combinations have been associated with the clinical condition (Omar 1966; Gourlay & Howard 1979; Stott *et al.* 1980; Thomas *et al.* 1981). However, not one of these microbial species alone produced the typical severe disease in normal or gnotobiotic animals (Howard *et al.* 1976; Gourlay *et al.* 1976*b*; Thomas *et al.* 1977). Only the inoculation of homogenates of the lungs of animals that have suffered the disease reproduced the severe clinical condition (Gourlay *et al.* 1976*a*; Thomas *et al.* 1981).

Periodontal disease and abdominal abscess are two human conditions with a mixed microbial aetiology that have been investigated by using model infections in experimental animals. MacDonald *et al.* (1960) used subcutaneous infection in the groin of guinea pigs to simulate invasion of sub-mucosal tissue in periodontal disease. Four oral bacteria, *Bacteroides melaninogenicus*, another bacteroides species, a motile Gram-negative rod and a facultatively anaerobic diphtheroid, were all required for successful infection. In a recent use of the same model (Mayrand & McBride 1980) *Klebsiella pneumoniae* and other 'helper' bacteria-prompted infections with *Bacteriodes asaccharolyticus*. Models for human abdominal abscesses have been established in mice, rats and guinea pigs (Wilkins 1979; Tally 1979; Bartlett & Onderdonk 1979; Kelly 1978), and all demonstrated synergy between faecal organisms in the production of abdominal abscesses. The rat model used by Bartlett, Onderdonk and their colleagues (Tally 1979; Bartlett & Onderdonk 1979) is typical. A gelatin capsule containing barium sulphate and rat caecal contents was inserted into the peritoneum of rats. All the animals developed peritonitis with bloodstream invasion by *E. coli* and about 40 % mortality; later abscesses developed in 100 % of the surviving animals. Treatment with gentamicin, which acts only against aerobic bacteria, reduced initial mortality but not subsequent abscess formation; on the other hand, clindamycin, which is inactive against coliforms, left initial mortality unaffected but prevented subsequent abscess formation. Thus, it appeared that coliforms were responsible for early mortality while anaerobes were critical to the development of abscesses. Further experiments used the gelatin capsule, barium sulphate, autoclaved rat caecal contents and single and mixed pure cultures of *E. coli*, an enterococcus and the anaerobes *Bacteroides fragilis* and *Fusobacterium varium*. When used singly, only *E. coli* produced mortality and none of the four species produced

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abscesses. Studies with all possible combinations of the four species showed that *E. coli* was essential for rapid mortality and that abscess formation required either *E. coli* or the enterococcus plus *B. fragilis* or *F. varium*. Abscesses were not formed by combinations of either the two facultative organisms or the two anaerobes (Tally 1979). A similar model in rats but with the use of human stool contents in the capsules, supported the role of *B. fragilis* in abscess formation, but, unlike the above experiments, indicated that it could contribute to the early deaths (Bartlett & Onderdonk 1979). Turning to a different animal model, *B. fragilis* and *E. coli* showed synergism in infection of surgical incisions in guinea pigs (Kelly 1978). Hence, there is little doubt that abdominal abscesses are caused by synergism between intestinal organisms that escape during trauma and that *B. fragilis* is an important member of the relevant mixture. The fact that 70–80 % of all clinical isolates from the *B. fragilis* group are capsulated (Bartlett & Onderdonk 1979) relates to its probable role in the synergy (see below).

TABLE 1. THE MULTIFACTORIAL NATURE OF PATHOGENICITY (VIRULENCE)

virulence determinants	strains...	virulent	less or not virulent				
A		A	–	A	A	A	A
B		B	B	–	B	B	B
C		C	C	C	–	C	C
D		D	D	D	D	–	D
E		E	E	E	E	E	–

(*a*) *Mechanisms of synergy between non-pathogenic or weakly pathogenic microorganisms*

The mechanisms of synergy arise from the multifactorial nature of pathogenicity (virulence). As stated before, to be pathogenic a microorganism must be able to infect mucous surfaces, penetrate into the tissues, grow *in vivo*, interfere with host defence mechanisms and damage the host. The biochemical bases of these essential steps in the disease process are the determinants of pathogenicity (Smith 1978). Since there are five different steps and each is complex, pathogenicity or virulence depends on the production of a number of different determinants. Loss of the ability to produce one or more of these determinants leads to partial or complete loss of virulence because one or more of the essential steps in disease production cannot be accomplished efficiently. Thus, as shown in table 1, loss of virulence would occur if any one of five different hypothetical determinants (A, B, C, D and E, corresponding, for example, with the five steps of disease production) were not produced, despite the fact that any avirulent or attenuated strain could form the other four determinants. This possession of virulence determinants by avirulent or attenuated strains means that if the missing determinant can be supplied or substituted by an outside source, virulence will be restored. The outside source might be another strain or another bacterial species also deficient in virulence determinants. The missing determinants might be supplied by complementation between the two organisms or by one or other of the pair producing a substitute for the missing determinant of the other. In the relatively few cases that have been investigated, it appears that such mechanisms may explain the synergy between non-pathogenic or weakly pathogenic bacteria.

The pathogenicity of a mixture of *F. necrophorus* and *C. pyogenes* in heel abscess in sheep appears to depend on an enhancement of the ability of *F. necrophorus* to grow in the host tissues by a growth stimulant derived from *C. pyogenes* and also on the inhibition of phagocyte defences

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by a toxic metabolite from the former, which prevented the ingestion and destruction of the latter as well as of itself (Roberts 1969). In the guinea-pig model for periodontal disease (see above), the diphtheroid was shown to produce vitamin K, an essential growth factor for *B. melanogenicus*. Similarly, in mixed infections in guinea pigs *K. pneumoniae* appeared to promote the establishment of *B. asaccharolyticus* by producing succinate, which could substitute for the haemin requirement of the anaerobe; indeed *K. pneumoniae*, and other succinate-producing 'helper' organisms active in the mixed infection, could be replaced by either succinate or haemin immobilized in agar (Mayrand & McBride 1980). Finally, the role of *B. fragilis* in the formation of abdominal abscesses appears to be in correcting a deficiency of other members of the intestinal flora in the ability to resist host defences, probably the action of phagocytes. The capsule of *B. fragilis* is composed of a polysaccharide that interferes with the action of phagocytes (Tally 1979; Bartlett & Onderdonk 1979; Quie *et al.* 1981) and, like live *B. fragilis* in the rat model described above, both killed *B. fragilis* and the purified polysaccharide promoted abscess formation in association with other intestinal organisms (Tally 1979; Bartlett & Onderdonk 1979).

Conclusion

The role of microbial interactions in infectious disease is being increasingly recognized both as regards protection and potentiation. There is some information on the mechanisms of these interactions, but much remains to be learned.

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