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Enhancement of the infectivity of *Fusobacterium necrophorum* by other bacteria

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SUMMARY

Necrobacillosis is caused by *Fusobacterium necrophorum* (FN), but other organisms are often present in the lesions. Their possible role was studied in experiments made with a virulent FN strain which, by itself, produced fatal necrobacillosis in mice provided that large doses ($>10^6$ organisms, subcutaneously) were given. Mice were inoculated subcutaneously with FN suspended in sub-lethal doses (0·1 ml) of undiluted or diluted broth cultures of other bacteria. Undiluted culture of a strain of *Escherichia coli* reduced the infective dose of FN to <10 organisms: in the necrobacillosis lesions that developed, fusobacteria greatly outnumbered *E. coli*. A heat-killed preparation or sterile filtrate of *E. coli* culture had little if any effect on FN. *Citrobacter freundii* and comparatively small numbers of *Corynebacterium* (*Actinomyces*) *pyogenes* produced effects similar to that of *E. coli*. An α-haemolytic streptococcus, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Fusobacterium nucleatum* also enhanced the infectivity of FN, though less strikingly than *E. coli*. FN increased the persistence *in vivo* of the α-haemolytic streptococcus and *B. fragilis*, and enabled the latter to multiply profusely.

INTRODUCTION

Fusobacterium necrophorum, a normal inhabitant of the gut of herbivores and other animals, gives rise to necrobacillosis via epithelia already damaged by traumatizing agents, maceration, bacteria, or viruses. The lesions (coagulative necrosis, and sometimes abscesses) occur on the external body surfaces or mucosae, and may spread haematogenously from such sites to the viscera. The many species affected include agricultural animals such as cattle, sheep, goats, horses, pigs, rabbits, and poultry (1); free-living and captive wild animals such as deer, antelope, and macropods (2); and man (3).

Some necrobacillosis lesions, for example a proportion of those that occur in the liver of cattle, yield pure growths of *F. necrophorum*. The majority, however, yield mixtures of bacterial species including *F. necrophorum*. The latter is the main pathogen and the role played, if any, by the other organisms is for the most part uncertain. Studies on foot infections in sheep revealed, however, a synergistic

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relationship between *F. necrophorum* and *Corynebacterium pyogenes* (4). These two organisms, injected together into the skin of sheep or guinea-pigs, produced lesions more severe than those caused by either alone; and histological examination showed that the proliferation of each organism was increased by the presence of the other.

Experimental studies in mice have also given information on synergistic relationships between *F. necrophorum* and other bacteria. Such studies have been based on abscess formation in the liver and elsewhere after intravenous or intraperitoneal inoculation (5–7), or on the production of local lesions by inocula given subcutaneously (8, 9). It would appear, however, that no quantitative examination has been made of the effect of other bacteria on the minimum infective dose of *F. necrophorum*. This is an unfortunate omission; the striking necrotizing activity of which *F. necrophorum* is capable – regardless of the presence or absence of other bacteria – is dependent for its expression on the initiation of infection, a process that probably requires an auxiliary mechanism. Thus fatal necrobacillosis is readily produced in experimental animals by the subcutaneous injection of pure cultures of a virulent strain of *F. necrophorum*, such as the one described in the present study, but large doses ($>10^6$) of viable organisms are needed. Smaller doses have no apparent effect.

The question of whether the minimum infective dose of *F. necrophorum* can be reduced by intercurrent infection with other bacteria, in particular those found in animal environments, forms the subject of this paper.

MATERIALS AND METHODS

The mice, culture media, anaerobic methods and viable count technique were essentially as already described (10).

Organisms

F. necrophorum strain A42, isolated from a wallaby with necrobacillosis, has been used extensively in laboratory experiments (10–13). An 18 h culture of this strain in BM broth (14) is, when administered subcutaneously to mice in a dose of 0·1 ml, invariably fatal; a 1 in 10 dilution is fatal to some but not all mice; and a 1 in 100 dilution is usually without apparent effect.

The other organisms used all belonged to species likely to be found in animal environments. Strains of *Citrobacter freundii* and *Corynebacterium* (*Actinomyces*) *pyogenes* were uterine isolates from cases of bovine endometritis. The α-haemolytic streptococcus came from the apparently normal uterus of a cow 4 days after calving, and *Bacteroides fragilis* strain A46 (10) from a wallaby with necrobacillosis. These strains, all of which originated from mixed infections, had undergone fewer than 10 subcultures since isolation.

The National Collection of Type Cultures supplied *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662, and *Fusobacterium nucleatum* NCTC 10562. The number of laboratory subcultures undergone by these strains was unknown.

Dual infection experiments

E. coli, *Cit. freundii*, *Coryne. pyogenes*, an α-haemolytic streptococcus, *Ps. aeruginosa*, *B. fragilis* and *F. nucleatum* were tested in turn for a possible

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Table 1. *Dual infection with* Fusobacterium necrophorum *and* Escherichia coli

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups of four given FN diluted in EC, itself diluted 1 in			
	1*	10	10^2	10^3
10^2	4	4	1	0
10^3	4	3	0	0
10^4	4	2	0	0
10^5	4	1	0	0
10^6†	3	0	0	0

FN, *F. necrophorum* culture; EC, *E. coli* culture.

* Viable count of undiluted EC = 407×10^6/0·1 ml.

† Dose of *F. necrophorum*/mouse = 100 viable organisms.

Controls: 12 mice inoculated with 0·1 ml of undiluted EC became infected but recovered: of 12 mice given 0·1 ml of a 1 in 100 dilution of FN in sterile diluent, 11 remained healthy and 1 died.

synergistic relationship with *F. necrophorum* strain A42. The purpose was to determine whether the infectivity of *F. necrophorum* was increased by the presence of the second organism and, if possible, whether *F. necrophorum* exerted any reciprocal effect.

Mice were inoculated subcutaneously on the outer aspect of the right thigh, in dose volumes of 0·1 ml, with 18 h cultures grown in BM broth. Test mice received decimal dilutions of *F. necrophorum* culture prepared in a diluent consisting of a neat culture of the organism under examination, or in an appropriate dilution (in sterile BM) thereof. Control mice received either an appropriate dilution (in sterile BM) of *F. necrophorum* culture alone, or neat culture of the organism under examination.

The experiments were assessed by two methods. (1) Mice were observed for the occurrence of severe and progressive necrobacillosis. Actively multiplying *F. necrophorum* was always present in such lesions, the first clinical sign of which was lameness. As these lesions were inevitably fatal, affected mice were killed to prevent suffering. (2) Bacterial multiplication *in vivo* was examined. After depilation, the right hind leg with part of the flank was removed, immersed for 2–3 s in boiling water, and transferred to a sterile Petri dish. With sterilized instruments a small piece of tissue (often 0·1–0·2 g) was removed from the lesion and weighed. After adding 1 ml of sterile BM medium this material was homogenized in a Griffith tube. A viable count (anaerobic, aerobic or both, as appropriate) of the homogenate was then made. Because the number of living bacteria probably varied considerably from one part of the lesion to another, caution was exercised in drawing conclusions from the viable counts.

RESULTS

Dual infection with F. necrophorum *and* E. coli

Table 1 shows that, though *E. coli* (407×10^6 organisms) alone was sub-lethal and *F. necrophorum* (10^6 organisms) alone virtually so, inocula containing both *F. necrophorum* ($\geqslant 100$ organisms) and *E. coli* (407×10^6 organisms) almost always

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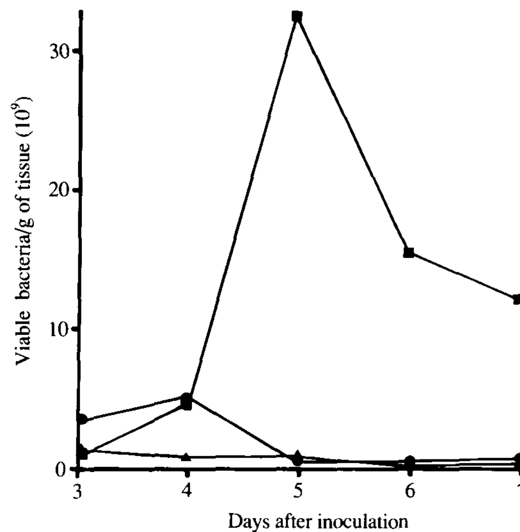

Fig. 1. Bacterial counts in the lesions produced by dual infection with *F. necrophorum* (170 organisms) and *E. coli* culture (0·1 ml). Each point represents the mean value from two mice. The counts of *F. necrophorum* and *E. coli* in dually infected mice are represented by ■——■ and ▲——▲, respectively. The counts in mice infected with *E. coli* alone are represented by ●——●.

produced fatal necrobacillosis. *E. coli* was therefore capable of reducing the infective dose of *F. necrophorum* by a factor of $> 10^4$. *E. coli* in a dose of 41×10^6 (but not 4×10^6) also had a striking, though somewhat reduced, effect on the infectivity of *F. necrophorum*. It should be noted that *E. coli* (407×10^6 organisms) alone produced a subcutaneous infection lasting more than 7 days, with a comparatively mild local lesion that usually ulcerated before healing.

In a further experiment (Fig. 1) mice dually infected with *F. necrophorum* and *E. coli* were compared with those receiving *E. coli* alone. Pairs of mice were killed at intervals to enable viable counts of bacteria in the lesions to be made. *F. necrophorum* multiplied profusely from an initial dose of 170 organisms, reaching a maximum count of *c.* 32×10^9/g of tissue 5 days after inoculation. The counts of *E. coli* in both groups of mice remained comparatively low, but infection was still present 7 days after inoculation. As before, *E. coli* reduced the infective dose of *F. necrophorum* by a factor of $> 10^4$ but, for reasons given under *Materials and Methods*, the experiment is not considered to have shown whether the *E. coli* infection was influenced by the presence of fusobacteria.

Dual infection with F. necrophorum *and* Cit. freundii

Table 2 shows that *Cit. freundii* had an effect similar to that of *E. coli* (Table 1) in enhancing the infectivity of *F. necrophorum*. Inocula of *Cit. freundii* alone, like those of *E. coli* alone, produced sub-lethal infection of the subcutaneous tissues of mice; but when even very few (160) *F. necrophorum* organisms were added fatal necrobacillosis resulted.

In a further experiment mice received inocula of *Cit. freundii* (140×10^6

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Table 2. *Dual infection with* Fusobacterium necrophorum *and* Citrobacter freundii

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups of four given FN diluted in CF, itself diluted 1 in		
	1*	10	10^2
10^2	4	3	0
10^3	4	1	0
10^4	4	0	0
10^5	2	0	0
10^6†	3	1	0

FN, *F. necrophorum* culture; CF, *Cit. freundii* culture.
* Viable count of undiluted CF = $80 \times 10^6/0·1$ ml.
† Dose of *F. necrophorum*/mouse = 160 viable organisms.
Controls: 12 mice inoculated with 0·1 ml of undiluted CF became infected but recovered: 12 mice given 0·1 ml of a 1 in 100 dilution of FN in sterile diluent remained healthy.

organisms) either alone or mixed with *F. necrophorum* (230 organisms). Pairs of mice infected by each type of inoculum were killed 4, 5 and 6 days (Fig. 2) later. Viable counts (millions/g) of organisms in diseased tissue ranged from 404–3575 (mean 1128) in mice given *Cit. freundii* alone; and from 41–535 (mean 216) *Cit. freundii* and 2923–34280 (mean 15371) *F. necrophorum* in dually infected mice. Thus the numbers of *Cit. freundii* were much smaller than those of *F. necrophorum*. For reasons given under *Materials and Methods* the experiment is not considered to have shown an unequivocal difference between the numbers of *Cit. freundii* in singly and dually infected mice.

Dual infection with F. necrophorum *and* Coryne. pyogenes

Table 3 shows that *Coryne. pyogenes* strongly enhanced the infectivity of *F. necrophorum*. A dose containing 110 fusobacteria was enabled, by the inclusion of 40×10^6 corynebacteria, to produce necrobacillosis in all inoculated mice; and, by the inclusion of only 4×10^6, in almost all. Even $0·4 \times 10^6$ corynebacteria enhanced to some degree the infectivity of *F. necrophorum*.

Coryne. pyogenes (40×10^6 organisms) alone produced a small nodular lesion containing viable organisms which, in a few mice but not in the majority, persisted for at least 12 days; and the viable counts (millions/g) of corynebacteria in the lesions ranged from 0·1–21800 (mean 11079) in four mice killed 4–5 days after inoculation. In 12 dually infected mice killed 4–7 days after inoculation the counts (million/g of diseased tissue) of corynebacteria and fusobacteria were, respectively, 144–7634 (mean 1850) and 2674–20167 (mean 12264).

Dual infection with F. necrophorum *and an α-haemolytic streptococcus*

Table 4 shows that the streptococcus (50×10^6 but not 5×10^6 organisms) enabled *F. necrophorum* (2×10^6 organisms) to produce necrobacillosis in 43% of inoculated mice. The corresponding figure for mice receiving the same dose of streptococci but only $\leqslant 200000$ *fusobacteria* was $\leqslant 20$%. Thus the ability of the streptococcus to assist the infectivity of *F. necrophorum*, though unequivocal, was

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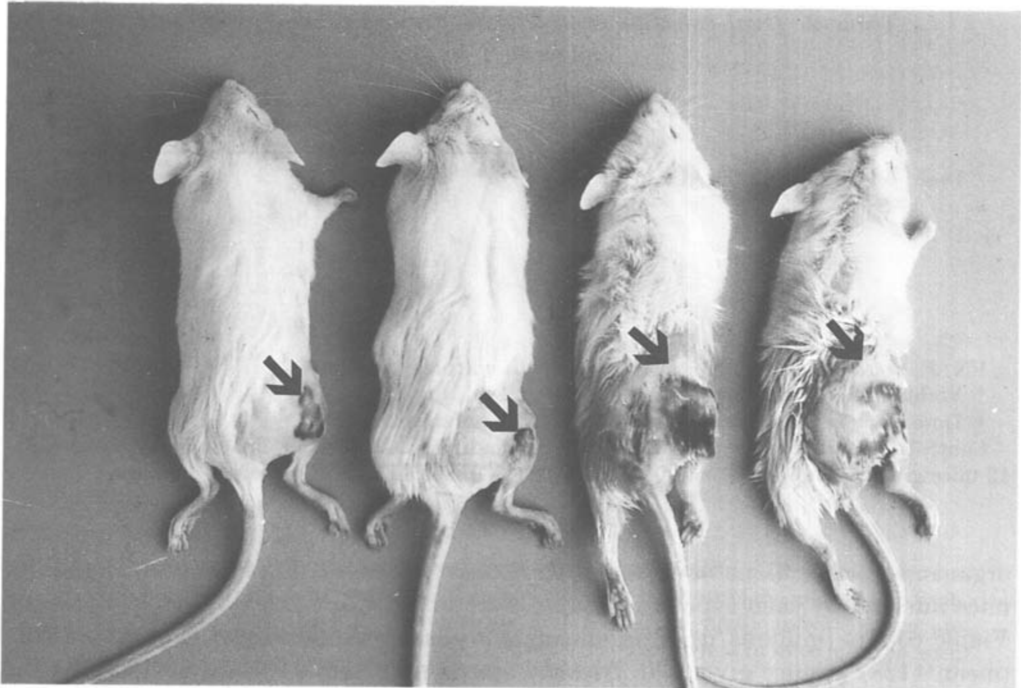

Fig. 2. The effect of *Cit. freundii* on the infectivity of *F. necrophorum*. Four mice were killed 6 days after inoculation. The two on the left received *Cit. freundii* (140×10^6 organisms) alone: this produced only a mild infection with a small ulcerating lesion. The two mice on the right received in addition a minute dose of *F. necrophorum* (230 organisms), which was enabled by the *Cit. freundii* to produce potentially fatal necrobacillosis. The area surrounding the inoculation sites have been depilated and the boundary of each lesion is marked (arrow).

Table 3. *Dual infection with* Fusobacterium necrophorum *and* Corynebacterium pyogenes

	Mice with necrobacillosis in groups of four given FX diluted in CP, itself diluted 1 in				
Dose: 0·1 ml of FX diluted 1 in	1*	10	10^2	10^3	10^4
10^2	4	3	3	1	1
10^3	4	3	2	0	—
10^4	4	4	0		—
10^5	4	4	1	—	—
10^6†	10‡	3	1	—	—

FX, *F. necrophorum* culture: CP, *Coryne. pyogenes* culture.

* Viable count of undiluted CP = 40×10^6/0·1 ml.

† Dose of *F. necrophorum*/mouse = 110 viable organisms.

‡ Group contained 10 mice.

Controls: 12 mice inoculated with 0·1 ml of undiluted CP all survived with minimal local lesions: of 12 mice given 0·1 ml of a 1 in 100 dilution of FX in sterile diluent, 10 remained healthy and 2 died: 12 mice given 0.1 ml of a 1 in 1000 dilution of FX in sterile diluent remained healthy.

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Table 4. *Dual infection with* Fusobacterium necrophorum *and an α-haemolytic streptococcus*

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups given FN diluted in AHS, itself diluted 1 in	
	1*	10
10^2†	13/30	0/12
10^3	4/20	—
10^4	1/6	—

FN, *F. necrophorum* culture; AHS, α-haemolytic streptococcus culture.
* Viable count of undiluted AHS = 50×10^6/0·1 ml.
† Dose of *F. necrophorum*/mouse = 2×10^6 viable organisms.
Controls: 12 mice inoculated with 0·1 ml of undiluted AHS, and 12 given 0·1 ml of a 1 in 100 dilution of FN in sterile diluent, remained healthy.

Table 5. *Synergy between* Fusobacterium necrophorum *and an α-haemolytic streptococcus as shown by counts of bacteria in lesions*

Number of mice	Dose (10^6) AHS	Dose (10^6) FN	Period (days) between inoculation and slaughter	Counts (10^6)/g of tissue AHS	Counts (10^6)/g of tissue FN
4	50	0	3	0	—
1	50	2	5	27	24488
1	50	2	5	30	16650
1	50	0·2	5	3	23550
1	50	0·2	5	18	18007

FN, *F. necrophorum*; AHS, α-haemolytic streptococcus.
Controls: 12 mice inoculated with 50×10^6 AHS, and 12 given 2×10^6 FN, remained healthy.

much less striking than that of *E. coli*, *Cit. freundii*, or *Coryne. pyogenes* (Tables 1–3).

The streptococcus (50×10^6 organisms) alone, which produced no obvious lesion, was completely eliminated from the tissues within 3 days (Table 5). This fortuitous circumstance enabled the experiment to show that *F. necrophorum* exerted a reciprocal effect. Thus in four dually infected mice killed 5 days after inoculation the streptococcus was present in the necrobacillosis lesions in numbers (millions/g of tissue) that ranged from 3–30 (mean 19). In the same mice the corresponding figures for *F. necrophorum* were 16650–24488 (mean 20673).

Dual infection with F. necrophorum *and* Ps. aeruginosa

In a preliminary experiment undiluted *Ps. aeruginosa* culture alone produced fatal infections in 8 of 10 mice. As a result culture was used subsequently at a dilution of at least 1 in 10.

Table 6 shows that *Ps. aeruginosa* (33×10^6 organisms) enabled *F. necrophorum* ($\geqslant 0{\cdot}17 \times 10^6$ organisms) to produce necrobacillosis in almost all mice inoculated. The ability of the pseudomonad to assist the infectivity of *F. necrophorum* was

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Table 6. *Dual infection with* Fusobacterium necrophorum *and* Pseudomonas aeruginosa

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups of four given FN diluted in PA, itself diluted 1 in			
	10*	10^2	10^3	10^4
10^2	4	1	1	0
10^3†	3	0	0	0
10^4	0	0	0	—
10^5	1	0	0	—
10^6	1	0	0	—

FN, *F. necrophorum* culture; PA, *Ps. aeruginosa* culture.
* Viable count of PA diluted 1 in 10 = 33×10^6/0·1 ml.
† Dose of *F. necrophorum*/mouse = $0·17 \times 10^6$ viable organisms.
Controls: 12 mice inoculated with 0·1 ml of a 1 in 10 dilution of PA in sterile diluent remained healthy; of 12 mice given 0·1 ml of a 1 in 100 dilution of FN in sterile diluent, 11 remained healthy and 1 died.

Table 7. *Dual infection with* Fusobacterium necrophorum *and* Bacteroides fragilis

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups given FN diluted in neat BF*
10^2†	10/22
10^3	5/22
10^4	0/6

FN, *F. necrophorum* culture; BF, *B. fragilis* culture.
* Viable count of neat BF = 800×10^6/0·1 ml.
† Dose of *F. necrophorum*/mouse = $1·5 \times 10^6$ viable organisms.
Controls: 12 mice inoculated with 0·1 ml of undiluted BF, and 12 given 0·1 ml of a 1 in 100 dilution of FN in sterile diluent, remained healthy.

thus somewhat more striking than that of the α-haemolytic streptococcus (Table 4).

Mice given *Ps. aeruginosa* (33×10^6 organisms) alone suffered only sub-lethal infections; the numbers of bacteria (millions/g of infected tissue) in three such animals killed 5–6 days after inoculation ranged from 0–607 (mean 154). In three mice given *F. necrophorum* ($0·17 \times 10^6$ organisms) in addition to *Ps. aeruginosa* (33×10^6 organisms) and killed 5–6 days later the counts (millions/g of diseased tissue) of the two bacterial species were, respectively, 281–5037 (mean 2181) and 0·2–1·7 (mean 0·7).

Dual infection with F. necrophorum *and* B. fragilis

Table 7 shows that *B. fragilis* (800×10^6 organisms) enabled *F. necrophorum* ($1·5 \times 10^6$ organisms) to produce necrobacillosis in 45% of inoculated mice. The corresponding figure for mice receiving the same dose of *B. fragilis* but only $0·15 \times 10^6$ fusobacteria was 23%. Thus the ability of *B. fragilis* to assist the infectivity of *F. necrophorum* was unequivocal but undramatic.

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Table 8. *Synergy between* Fusobacterium necrophorum *and* Bacteroides fragilis *as shown by counts of bacteria in the lesions*

Number of mice	Dose (10^6)		Period (days) between inoculation and slaughter	Counts (10^6)/g of tissue	
	BF	FN		BF	FN
4	425	0	3	0*	—
1	425	0·1	5	15441	27552
1	425	0·1	6	13416	21083
1	425	0·1	6	8585	39981
1	425	0·1	6	17223	43842
1	425	0·1	6	9407	23518

FN, *F. necrophorum*; BF, *B. fragilis*.
* Three mice were free from infection and one yielded 15 BF colonies from a large inoculum.
Controls: 12 mice inoculated with 425×10^6 BF, and 12 given 10^6 FN, remained healthy.

Table 9. *Dual infection with* Fusobacterium necrophorum *and* Fusobacterium nucleatum

Dose: 0·1 ml of FN diluted 1 in	Mice with necrobacillosis in groups given FN diluted in FNU, itself diluted 1 in	
	1*	10
10^2	15/20	1/12
10^3	4/20	—
10^4†	1/6	—

FN, *F. necrophorum* culture; FNU, *F. nucleatum* culture.
* Viable count of undiluted FNU = 200×10^6/0·1 ml.
† Dose of *F. necrophorum*/mouse = 10000 viable organisms.
Controls: 12 mice inoculated with 0·1 ml of undiluted FNU, and 12 given 0·1 ml of 1 in 100 dilution of FN in sterile diluent, remained healthy.

In a further experiment (Table 8) *B. fragilis* (425×10^6 organisms) alone, which produced no obvious lesions, was usually eliminated from the tissues within 3 days. As in an earlier experiment (Table 5) this fortuitous circumstance revealed that in dual infections *F. necrophorum* exerted a reciprocal effect on *B. fragilis*. Thus in five mice killed 5–6 days after dual infection *B. fragilis*, like *F. necrophorum*, was present in the lesions of necrobacillosis in numbers that indicated profuse multiplication.

Dual infection with F. necrophorum *and* F. nucleatum

Table 9 shows that undiluted *F. nucleatum* culture (200×10^6 organisms) enabled 10^6 *F. necrophorum* to produce necrobacillosis in 75% of inoculated mice. The corresponding figure for mice receiving the same dose of *F. nucleatum* but only $0{\cdot}1 \times 10^6$ *F. necrophorum* was 20%. Thus *F. nucleatum* resembled *B. fragilis* in assisting the infectivity of *F. necrophorum* to a degree that was unequivocal but not striking.

Difficulty in distinguishing between the colonies of *F. necrophorum* and

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F. nucleatum prevented any assessment of the numbers of the two organisms in the lesions.

The effect of E. coli. Cit. freundii *and* Coryne. pyogenes *culture filtrates on the infectivity of* F. necrophorum

Sterile membrane filtrates of broth cultures of the three facultative anaerobes were prepared. Each of the three fitrates was injected into four mice in doses of 0·1 ml in which were suspended $1·7 \times 10^6$ *F. necrophorum*. These 12 inoculated mice remained healthy. At the same time each of the three 'parent' whole cultures was injected into four mice in doses of 0·1 ml in which were suspended only 170 *F. necrophorum*. Necrobacillosis developed in 11 of these 12 mice. The filtrates therefore lacked the ability of the whole cultures to enhance the infectivity of *F. necrophorum*.

The effect of killed whole culture of E. coli *on the infectivity of* F. necrophorum

Twelve mice were each inoculated with 0·1 ml of live *E. coli* broth culture in which was suspended a minute dose (eight organisms) of *F. necrophorum*. Necrobacillosis developed in all these mice. At the same time doses of *F. necrophorum* ranging in decimal dilutions from 85000 to eight organisms were suspended in 0·1 ml volumes of the *E. coli* culture killed by heating at 60 °C for 30 min in a waterbath: these doses were then administered to mice in five groups of six. All 30 animals survived except two which died from necrobacillosis in the group that received 85000 *F. necrophorum*. If the killed *E. coli* culture possessed any ability to enhance the infectivity of *F. necrophorum* it was therefore negligible by comparison with that of the living culture, which enabled < 10 *F. necrophorum* organisms to produce fatal necrobacillosis in 100% of mice.

DISCUSSION

F. necrophorum is well capable of producing fatal experimental necrobacillosis by subcutaneous inoculation without assistance from other bacteria, provided that the large dose invariably needed to initiate infection is given. The experiments described in this report show, however, that the minimum infective dose is readily reduced by the presence of other bacteria. All of seven bacterial species tested produced such an effect, though to a degree that varied greatly.

Thus the infective dose of the *F. necrophorum* strain used was reduced from $> 10^6$ to < 10 organisms by suspension in 0·1 ml of undiluted *E. coli* culture, which by itself produced only a sub-lethal infection. The dual infection produced fatal necrobacillosis with fusobacterial proliferation that greatly outstripped the growth of *E. coli* in the lesions. The infectivity-enhancing effect of *E. coli* was reduced when the culture was diluted 1 in 10, and virtually abolished at 1 in 100. A heat-killed preparation or sterile filtrate of undiluted *E. coli* culture had little if any effect on *F. necrophorum*.

In comparing the effect produced by *E. coli* with that of the six other organisms tested the variations in viable count of the undiluted cultures must be borne in mind. It would seem, however, that *Cit. freundii* produced an effect similar to that of *E. coli*: and *Coryne. pyogenes* was also strikingly active, even in a comparatively

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small dose. The remaining organisms tested (an α-haemolytic streptococcus, *Ps. aeruginosa*, and the two anaerobes *B. fragilis* and *F. nucleatum*) were also active, but much less so.

In only two of the seven dual infections studied was the experimental design capable of showing that the presence of *F. necrophorum* affected the second organism. Thus because the α-haemolytic streptococcus and *B. fragilis*, by themselves, were quickly eliminated from the tissues it became clear that the presence of *F. necrophorum* increased their persistence *in vivo* (Tables 5 and 8); it also enabled *B. fragilis* to proliferate dramatically.

The mechanism by means of which the minimum infective dose of *F. necrophorum* was reduced in the presence of other bacteria is unknown. It would seem, however, that because enhancement of infectivity was brought about by strikingly disparate organisms (varying from strictly aerobic to facultatively or strictly anaerobic) and to strikingly different degrees, the mechanism probably varied. It may be relevant that dilution of *F. necrophorum* cultures, with consequent increased separation of fusobacterial cells from each other, has an adverse effect on infectivity (12).

Many animals live in close contact with their own faeces, and *F. necrophorum*, the main causative agent of necrobacillosis, is an inhabitant of the gut. The bacterial species that frequently accompany *F. necrophorum* in necrotic lesions include many that also arise from the gut, and others that inhabit the skin and mucous membranes (15). The work described here suggests that these subsidiary agents play an important role in the initiation of *F. necrophorum* infection.

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