Technical method

```

2240IF A=13 THEN 2290
2250A$=CHR$(A)
2260IF A$="M" OR A$="m" THEN 2290
2270IF A$<>"C" AND A$<>"c" THEN 2230
2280Box=1:Labls=2:Vals=2
2290PRINT TAB (2,23);"Enter max. count: (default=100) ";
2300INPUT Cmax%
2310IF Cmax%=0 THEN Cmax%=100:ENDPROC
2320ENDPROC
2330REM
2340REM -----
2350REM --- Display/erase warning message re display status
2360REM
2370DEF PROC Warn(Onoff)
2380VDU 4
2390IF Onoff=0 THEN 2420
2400PRINT TAB(3,15);"**** Counts display incomplete ****":Won=1
2410VDU 5:ENDPROC
2420PRINT TAB(37,15);
2430FOR Q=1 TO 35:VDU 127:NEXT Q
2440Won=0:VDU 5:ENDPROC

```

Editorial note

We are not sure we like publishing programs in the above format, but we are bowing to increasing pressures—tell us if it is helpful or not!

A cassette containing this program ready for use may be obtained from the department of haematology of the East Birmingham Hospital.

Letters to the Editor

Effect of *Bacteroides fragilis* on the phagocytic killing of *Escherichia coli*

We read with interest the article by Vel *et al*¹ published recently in your journal. Having re-examined the inhibitory effect of *Bacteroides fragilis* on the in vitro killing of *Escherichia coli* by human polymorphonuclear leucocytes, the authors report that such impairment occurred only when the *E coli* strain required intact complement for opsonophagocytosis. We believe this paper to be a useful contribution to the growing volume of published work on the potential for synergistic interaction between organisms in infection.

In their discussion of results Vel *et al* state that we² were unable to show any impairment of clearance of *E coli* from peritoneal cavities of mice after intraperitoneal inoculation of the coliform in the presence of any one of 10 strains of *B fragilis*. The authors suggest that the *E coli* strain we used might have been one of those not requiring complement for phagocytosis and killing.

Evidence that the above suggestion is not applicable to our findings comes from two sources. Firstly, in experimental studies described by Finlay-Jones *et al*³ complement components required for

phagocytosis and killing of *Proteus mirabilis* by mouse neutrophils in vitro were absorbed by *E coli*. That strain is the one used by Reznikov *et al*.² Secondly, in phagocytic killing assays the *E coli* strain in question is not killed either in the absence of serum or in the presence of heated serum (Table). Moreover, opsonisation of that *E coli* strain for phagocytic killing by mouse neutrophils will proceed in the presence of Mg⁺⁺ and EGTA but not edetic acid.

We regret that the above information does not account for the discrepancy between results derived in vitro¹ and those derived in vivo.² In the meantime we await the appearance of the article by Verweij-

van Vught *et al*,⁴ which describes a study in which, as indicated by Vel *et al*,¹ the *E coli* strain requiring complement for phagocytic killing in vitro also produced subcutaneous abscesses in mice because its clearance was impaired by the accompanying *B fragilis*. Differences between the latter results and ours² may relate to the host defence mechanisms operative at different sites.

JJ FINLAY-JONES

M REZNIKOV

Clinical Microbiology Unit,

School of Medicine, Flinders University,

and
Flinders Medical Centre,
Bedford Park, South Australia, 5042,
Australia

Requirement of serum for phagocytosis and killing of *Escherichia coli* by murine neutrophils*

Serum in phagocytic assay†	No of experiments	Phagocytic killing at 120 min‡
None	7	-0.45 ± 0.16
Heated NMS (50°C, 30 min)	5	0.06 ± 0.19
Heated NMS (56°C, 30 min)	6	-0.09 ± 0.10
NMS	7	2.51 ± 0.44

*Methods for preparing neutrophils and performance of the phagocytic killing assay are given elsewhere.³ To calculate phagocytic killing the change in the viable count in each tube during the 2 hour phagocytic killing assay was determined, and the value for the tube containing neutrophils was subtracted from that of the relevant tube without neutrophils.³

†NMS = normal mouse serum.

‡Units are log₁₀ CFU/ml (mean ± SEM). A negative value means that growth of the organism was better in the presence of neutrophils than in their absence.

References

- ¹ Vel WAC, Namavar F, Verweij-van Vught AMJJ, Pubben ANB, MacLaren DM. Killing of *Escherichia coli* by human polymorphonuclear leukocytes in the presence of *Bacteroides fragilis*. *J Clin Pathol* 1985;38:86-91.
- ² Reznikov M, Finlay-Jones JJ, McDonald PJ. Effect of *Bacteroides fragilis* on the peritoneal clearance of *Escherichia coli* in mice. *Infect Immun* 1981;32:398-9.
- ³ Finlay-Jones JJ, Hill NL, Nulsen MF, Pruul H, McDonald PJ. Opsonins in normal mouse serum for the phagocytic killing of *Proteus mirabilis* by murine neutrophils. *Br J Exp Pathol* 1984;65:711-8.
- ⁴ Verweij-van Vught AMJJ, Namavar F, Sparrius M, Vel WAC, MacLaren DM. Pathogenic synergy between *Escherichia coli* and *Bacteroides fragilis*: studies in an experimental mouse model. *J Med Microbiol* (in press).

Dr Vel and others reply as follows:

Inhibition of phagocytosis and killing of facultative bacteria by polymorphonuclear leukocytes in the presence of anaerobic bacteria is regarded as a possible mechanism by which mixed infections with these bacteria can develop.¹ As many other authors have done, we studied the inhibition of killing in vitro and the effects of anaerobes on the clearance of facultative bacteria in vivo.¹⁻¹⁰ In vitro we found inhibition of the killing of facultative bacteria by human polymorphonuclear leukocytes, provided this killing was dependent on complement.¹⁰ This inhibition could be achieved not only with anaerobes but also with other complement depleting organisms such as *Staphylococcus aureus* or Zymosan.¹⁰ In vivo, in a mouse skin model, we found reduction of the clearance of *Escherichia coli* when it was inoculated simultaneously with *Bacteroides fragilis*.⁸ We suggested that the failure to find such an effect by Reznikov *et al*⁹ might have been caused by their *E coli* strain not requiring complement for its killing. Their proof that the *E coli* strain which failed to maintain itself in the presence of *B fragilis* in an experimental infection does need complement to be killed in vitro rules that explanation out.

In our further experiments, the results of which are shown in the Table, *B fragilis* and *B vulgatus* behaved similarly in inhibiting the in vitro killing of *E coli* by polymorphonuclear leukocytes; however, in vivo *B fragilis* had an inhibiting effect on the clearance of *E coli* and *B vulgatus* did not. The presence or absence of synergy seems to be related to the virulence of

Comparison of *B fragilis* BE1 and *B vulgatus* BE16

	<i>B fragilis</i> BE1	<i>B vulgatus</i> BE16
<i>In vitro</i>		
Killing of <i>bacteroides</i> by human polymorphonuclear leukocytes: survival of an inoculum of 7.0 after 90 min*	5.4	6.6
Inhibition of the killing of <i>E coli</i> EB1 in the presence of <i>bacteroides</i> after 90 min†	32	50
Complement depletion by <i>bacteroides</i> ‡	93.3	90.2
<i>In vivo</i>		
Survival of <i>bacteroides</i> in monoinfections after three days (inoculum 8.3)*	6.4	2.0
Survival of <i>E coli</i> in the presence of <i>bacteroides</i> after six days†	2200	4.9

*log viable count

†Inhibitory index: viable count of *E coli* in the presence of *bacteroides* divided by the viable count of *E coli* alone

‡CH₅₀ in serum after absorption with *bacteroides* cells: percentage of control serum, treated with phosphate buffered saline.

Methods of *in vitro* experiments are described by Vel *et al*¹⁰ and those of *in vivo* experiments by Verweij-van Vught⁸

these strains in monoinfections and not to their in vitro killing, complement consumption, or inhibition of killing of *E coli*. We therefore conclude that the synergistic effect in vivo is dependent on the virulence of the "helper organism." Furthermore, the virulence of the *E coli* strain is also of critical importance, since in our hands only with doses within certain narrow ranges can the synergistic effect be seen, not only in our mouse skin model but even more so in an intraperitoneal mouse model (unpublished data).

We therefore believe that our recent results can explain those of Reznikov *et al*⁹: the ability to consume complement and to inhibit phagocytosis and killing in vitro is no guarantee of synergy in vivo. In our opinion, however, inhibitory effects on phagocytosis and killing may still play a crucial part in the pathogenesis of mixed infections, provided the individual bacteria are virulent enough.

WAC VEL
AMJJ VERWEIJ-VAN VUGHT
F NAMAVAR
DM MACLAREN
Vrije Universiteit,
Faculty of Medicine,
Department of Medical Microbiology and
Parasitology,
1007 MC Amsterdam
The Netherlands

References

- ¹ Ingham HR, Sisson PR, Tharagounet D, Selkon JB, Codd AA. Inhibition of

phagocytosis in vitro by obligate anaerobes. *Lancet* 1977;ii:1252-4.

- ² Namavar F, van Vught AMJJ, MacLaren DM. Bacterial synergy in mixed aerobic and anaerobic infections. *Lancet* 1980;ii:659.

- ³ Jones GR, Gemmel CG. Impairment by *Bacteroides* species of opsonisation and phagocytosis of enterobacteria. *J Med Microbiol* 1982;15:351-61.

- ⁴ Namavar F, Verweij AMJJ, Bal M, van Steenberg TJM, de Graaff J, MacLaren DM. Effect of anaerobic bacteria on the killing of *Proteus mirabilis* by human polymorphonuclear leukocytes. *Infect Immun* 1983;40:930-5.

- ⁵ Wade BH, Kasper DL, Mandell GL. Interactions of *Bacteroides fragilis* and phagocytes: studies with whole organisms, purified capsular polysaccharide and clindamycin-treated bacteria. *J Antimicrob Chemother* 1983;11 (suppl C):51-62.

- ⁶ Onderdonk AB, Bartlett JG, Louie T, Sullivan-Seigler N, Gorbach SL. Microbial synergy in experimental intra-abdominal abscess. *Infect Immun* 1976;13:22-6.

- ⁷ Kelly MJ. The quantitative and histological demonstration of pathogenic synergy between *Escherichia coli* and *Bacteroides fragilis* in guinea pig wound. *J Med Microbiol* 1978;11:513-25.

- ⁸ Verweij-van Vught AMJJ, Namavar F, Sparrius M, Vel WAC, MacLaren DM. Pathogenic synergy between *E coli* and *Bacteroides fragilis*: studies in an experimental mouse model. *J Med Microbiol*.

- ⁹ Reznikov M, Finlay-Jones JJ, MacDonald PJ. Effect of *Bacteroides fragilis* on the peritoneal clearance of *Escherichia coli* in mice. *Infect Immun* 1981;32:398-9.

- ¹⁰ Vel WAC, Namavar F, Verweij-van Vught AMJJ, Pubben ANB, MacLaren DM. Killing of *Escherichia coli* by human polymorphonuclear leukocytes in the presence of *Bacteroides fragilis*. *J Clin Pathol* 1985;38:86-91.