# AN INTERFERENCE PHENOMENON CAUSED BY *PASTEURELLA PESTIS*

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(With 4 Figures in the Text)

Probably the earliest account of an interference phenomenon was that described by Pasteur (1886). He found that normal dogs developed rabies when injected with small amounts of brain emulsion from rabid street dogs. Ten times the amount of brain emulsion made the dogs refractory.

Since then the phenomenon has been described in a number of other virus infection systems. Henle & Chambers (1941) found interference using influenza virus and the chick embryo, Vieuchange (1940) with vaccinia and the rabbit, and Green (1945) described it as occurring with ferret-modified distemper virus injections into young dogs. Boyd (1951) described an interference phenomenon occurring with *Salmonella typhi murium* and phage A 1.

Until quite recently the phenomenon had not been described with infective agents capable of growing in an extracellular environment. Evans & Perkins (1954) described an interference effect produced by the intracerebral injection of mice of a killed vaccine, or of a cell-free vaccine, at the same time as a normally lethal dose of *Haemophilus pertussis*.

This paper is an account of the production of an interference-like phenomenon by *Pasteurella pestis* when injected into the peritoneal cavity of the white laboratory rat.

## MATERIALS AND METHODS

*Cultures.* Various strains of *Past. pestis* were grown for 24 hr. at 28° C. in a tryptic digest meat broth containing 5 % of a peptic digest of sheep blood. These cultures were stored at 4° C. and used for subculture. The subcultures were made by adding 0·5 ml. of the stock strain to 50 ml. of the broth in a conical flask and incubating at 28° C. for 20 hr. with constant shaking. The titre of viable organisms so obtained was usually in the region of $2 \times 10^9$/ml.

*Experimental animals.* Small white laboratory rats weighing 40–60 g. were used. Each rat, after injection, was held in a separate cage, the animals being randomized prior to injection.

*Material injected.* Unless otherwise stated in the text the organisms injected were contained in phosphate buffer dilutions (pH 7·6) of the broth culture; 0·5 ml. amounts were injected intraperitoneally.

*Viable counts.* Viable counts were made on all the challenge cultures prior to injection. After counting the total number of organisms/ml. of culture, dilutions were made in buffer and 0·1 ml. amounts spread on tryptic meat digest peptic

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sheep blood agar plates which had been dried for 45 min. at 37° C. The plates were incubated for 48 hr. at 28° C. and the developing colonies counted.

## RESULTS

### *The production of interference*

Rats were injected with serial ten-fold dilutions of various strains of *Past. pestis*. Eight different strains were examined by intraperitoneal injection into fifty rats, ten at each of five dose levels. It was noticed that five of the eight strains were less rapidly fatal at a challenge dose of $10^6$ organisms than at a challenge dose of $10^3$ organisms. This suggested that an interference phenomenon was occurring and a selected virulent strain (L37) was therefore injected into a larger series of rats to find out whether the interference was significant.

Table 1. *The dose-death response of white rats injected intraperitoneally with* Pasteurella pestis L37 *cultured in broth at* 28° C.

| Challenge dose | No. of rats injected | Total no. of rats dead: Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| $5 \times 10^3$ | 50 | 0 | 0 | 29 | 50 | . | . | . | . | . |
| $5 \times 10^6$ | 50 | 0 | 3 | 6 | 18 | 34 | 41 | 47 | 49 | 49 |
| $5 \times 10^6$ | 10 | 0 | 0 | 0 | 3 | 6 | 8 | 8 | 9 | 10 |
| $7{\cdot}6 \times 10^7$ | 10 | 0 | 8 | 10 | 10 | 10 | . | . | . | . |

Table 2. *The interference phenomenon produced by thrice-washed cultures of* Pasteurella pestis L37, *injected intraperitoneally into white rats*

| Challenge dose | No. of rats injected | Total no. of rats dead: Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
|---|---|---|---|---|---|---|---|---|
| $6{\cdot}2 \times 10^2$ | 20 | 0 | 1 | 8 | 13 | 16 | 19 | 20 |
| $6{\cdot}2 \times 10^5$ | 20 | 0 | 0 | 3 | 6 | 12 | 15 | 15 |

The upper part of Table 1 shows an interference effect that was statistically significant when the challenge dose was increased from $5 \times 10^3$ to $5 \times 10^6$ organisms, but with a further increase to $7{\cdot}6 \times 10^7$ (lower part of same table) this effect had disappeared and the animals were dying at least as rapidly as with a dose of $5 \times 10^3$ organisms.

The suspensions used for these experiments were all buffer dilutions of a broth culture, but in order to eliminate the possibility that some protective substance was present in the culture fluid, a broth culture was centrifuged, washed three times in buffer and resuspended in buffer. Serial ten-fold dilutions of this suspension were made and injected into rats at two dose levels (Table 2).

Interference became more marked as the challenge was increased up to a critical level at which interference was overcome. This critical level varied from animal to animal because occasionally animals injected with $5 \times 10^6$ organisms died an acute death within 48 hr.

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*The production of interference by the addition of vaccines to virulent organisms*

The addition of killed organisms, of living avirulent organisms, or of a cell-free filtrate of a broth culture, interfered with the lethal action of a small challenge of virulent organisms (Tables 3, 4 and 5). The protective action of the killed organisms,

Table 3. *The production of interference by killed virulent organisms injected intraperitoneally at the same time as living virulent organisms*

Twenty-five rats injected with 0·5 ml. of each mixture. Organisms killed by heat or by ultra-violet irradiation.

| | | Total no. of rats dead | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose of viable L37 | Dose of killed L37 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
| $1{\cdot}5 \times 10^4$ | Nil | 0 | 2 | 21 | 25 | . | . | . | . |
| $2{\cdot}9 \times 10^6$ | Nil | 0 | 3 | 8 | 16 | 16 | 17 | 17 | 18 |
| $1{\cdot}5 \times 10^4$ | $2{\cdot}9 \times 10^6$ (U.V.L. killed) | 0 | 0 | 11 | 21 | 24 | 24 | 24 | 24 |
| $1{\cdot}5 \times 10^4$ | $2{\cdot}9 \times 10^6$ (heat killed) | 0 | 0 | 10 | 21 | 25 | . | . | . |

Table 4. *The protective action of a broth-cultured avirulent* Pasteurella pestis, *strain Tjiwidej, injected intraperitoneally at the same time as virulent* Past. pestis L37

Fifteen rats injected with each mixture.

| | | Total no. of rats dead | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dose of L37 | Dose of Tjiwidej | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
| $4 \times 10^3$ | $4 \times 10^3$ | 0 | 13 | 14 | 15 | . | . | . |
| $4 \times 10^3$ | $4 \times 10^6$ | 0 | 0 | 1 | 2 | 3 | 3 | 5 |
| $4 \times 10^3$ | $4 \times 10^7$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5. *The production of interference by a* 1 *in* 20 *cell-free filtrate of a* 20 *hr. broth culture of* Pasteurella pestis L37

Twenty rats were each challenged with $8{\cdot}7 \times 10^3$ *Past. pestis* L37 intraperitoneally suspended in a 1 in 20 culture filtrate. Twenty control rats received the same challenge dose suspended in buffer.

| | Total no. of rats dead | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 10 |
| Suspending fluid | | | | | | | |
| Phosphate buffer | 0 | 4 | 16 | 20 | . | . | . |
| Broth filtrate | 0 | 1 | 10 | 15 | 19 | 19 | 19 |

which were killed either by heating at 60° C. for 1 hr. or by exposure to a Hanovia germicidal lamp, was small, and corresponded to the degree of protection afforded by the culture filtrate. Marked interference was produced by the living virulent organisms used as a control (Table 3), but this was considerably less than that produced by the living avirulent strain Tjiwidej, at a dose of $4 \times 10^7$ organisms, when interference appeared to be complete (Table 4).

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*The production of non-specific interference*

Intravenous injection of Indian ink prior to an intraperitoneal challenge of virulent L37 afforded a considerable degree of protection to the rats (Table 6).

Intraperitoneal injection of heat-killed *Salmonella typhi murium* produced a non-significant interference on one occasion, but this was not repeatable.

*The multiplication of* Pasteurella pestis *within the rat body following intraperitoneal challenge*

In order to follow the fate of injected organisms, rats were injected with buffer dilutions of a culture of *Past. pestis* L37 grown at 28° C. Thirty rats received an interfering dose and thirty rats a smaller dose. At intervals five rats of each series were anaesthetized with chloroform and 2 ml. amounts of plain phosphate buffer were injected into the peritoneal cavities. The rats were then returned to the chloroform until dead. The thoraces of the rats were opened and blood taken from the left ventricle of the heart with a Pasteur pipette. The blood was taken from the heart prior to opening the peritoneum in order to prevent possible soiling of the pipette by plague organisms in the peritoneal cavity. The blood was placed into

Table 6. *The protective action of intravenous Indian ink against a challenge of* $6{\cdot}4 \times 10^3$ Pasteurella pestis L37

Small white rats injected with 0·2 ml. Indian ink 3 hr. prior to the intraperitoneal challenge dose.

| Intravenous injection | No. of rats injected | Total no. of rats dead | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
| Buffer | 8 | 0 | 4 | 6 | 8 | . | . | . | . |
| Ink | 8 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 3 |

tubes containing heparin and viable counts were made. The peritoneal cavities were opened and as much free fluid as possible removed. Viable counts were made on the fluids.

Although fluid was added to the peritoneal contents of the rats it was felt that this would not alter the curves of graphs of viable counts made on the fluids. These rats did not develop ascites.

The injection of $8 \times 10^3$ L37 intraperitoneally resulted in a very rapid phagocytosis of the organisms, more than 95% being removed. A slow increase in the number of organisms recoverable from the peritoneal cavities then occurred and after 24 hr. organisms were present in the blood stream. Within 48 hr. the increase in the number of organisms in the blood stream was more rapid than in the peritoneal cavity (Figs. 1, 2).

After a challenge with $1{\cdot}8 \times 10^6$ L37 an initial fall occurred in the number of recoverable organisms, but the striking difference was the early appearance (within $1\frac{1}{2}$ hr.) of organisms in the blood stream (Figs. 3, 4). Thereafter the number of recoverable organisms either increased or decreased slowly. This corresponded to the survival of some rats following an interfering dose.

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In another series of rats challenged with $2{\cdot}3 \times 10^7$ L 37 the same initial fall in the peritoneal content occurred. In this instance the subsequent multiplication of organisms in the peritoneal cavity overtook the blood-stream increase and some of the rats developed a turbid bacterial culture-like ascites.

## DISCUSSION

Virulent strains of *Past. pestis* were less lethal for rats at a dose of $10^6$ organisms than at $10^3$ organisms. This would appear to be analogous to the auto-interference phenomenon of the virus research workers. The production of virus interference is

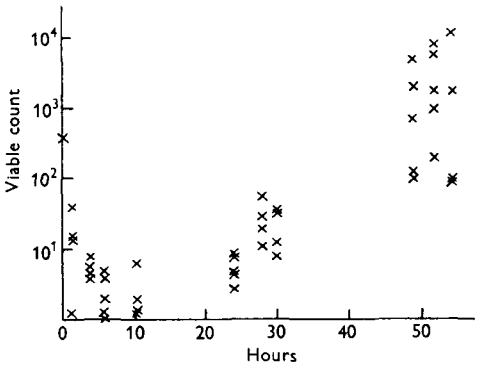


Fig. 1.

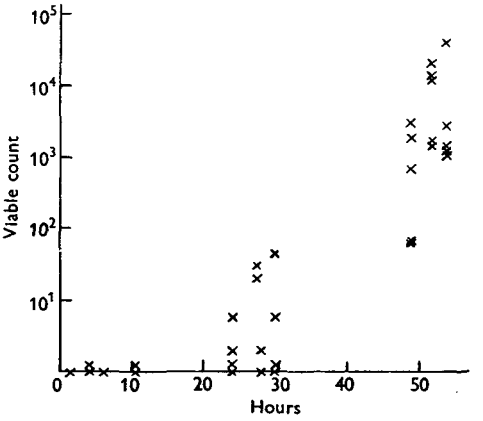


Fig. 2.

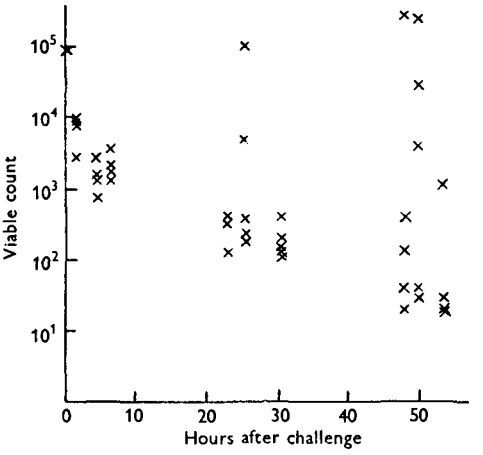


Fig. 3.

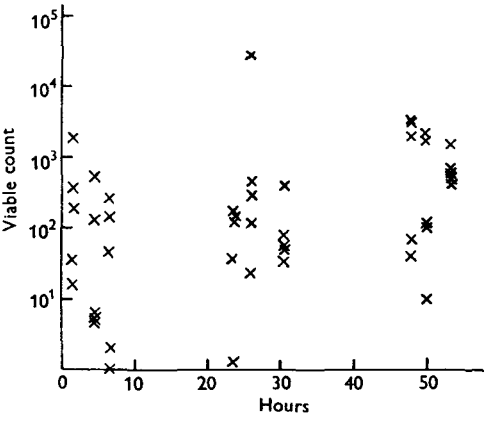


Fig. 4.

Fig. 1. The number of viable *Past. pestis* per 0·1 ml. peritoneal washing from rats killed at intervals following intraperitoneal challenge with $8 \times 10^3$ L 37.

Fig. 2. The number of viable *Past. pestis* per 0·1 ml. blood of rats killed at intervals following intraperitoneal challenge with $8 \times 10^3$ L 37.

Fig. 3. The number of viable *Past. pestis* per 0·1 ml. peritoneal washing from rats killed at intervals following intraperitoneal challenge with $1{\cdot}8 \times 10^6$ L 37.

Fig. 4. The number of viable *Past. pestis* per 0·1 ml. blood of rats killed at intervals following intraperitoneal challenege with $1{\cdot}8 \times 10^6$ L 37.

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generally held to be due to the presence of incomplete or inactive virus in the challenge competing with active particles for a favourable site for intracellular multiplication. *Past. pestis* almost certainly multiplies extracellularly, and it is therefore difficult to associate the methods by which interference is produced even though the addition of avirulent organisms to the challenge protected the rat completely.

A non-specific stimulation of the rat defences by the intravenous injection of Indian ink protected the animals to some extent. The addition of a cell-free filtrate of a broth culture to the challenge was partly effective in preventing rapid death. These two facts suggested that interference was not due to a limiting amount of a hypothetical growth factor preventing the multiplication of the interfering dose.

The blood and peritoneal contents of rats following a small challenge dose of strain L37 showed that the organisms did not primarily multiply in the peritoneal cavity. Histological examination of the livers of rats killed by a small challenge dose showed that the liver sinusoids were packed with clusters of organisms, many apparently phagocytosed by the Kupffer cells. A few small focal necroses were present. These focal necroses were more numerous in rats which had been challenged with an interfering dose and could be detected 24 hr. after challenge. It seems reasonable to suggest that these focal necroses are areas in which a hypersensitivity reaction has occurred associated with the killing of plague organisms by the reticulo-endothelial system in the liver. The interference was not produced by strain L37 grown at 37° C. even though the minimum LD50 of these organisms was significantly greater than when grown at 28° C.

No significant interference was produced in a number of experiments in which rats were injected subcutaneously, intracutaneously or intravenously with various strains of *Past. pestis*.

No bactericidal antibody, opsonin or complement increase was detected within 3 days in sera of rats challenged intraperitoneally with an interfering dose.

The phenomenon was not observed in mice or guinea-pigs following intraperitoneal injection of *Past. pestis*.

On the evidence presented it is possible that the type of interference phenomenon that has been observed may be partly explained as resulting from an intensive stimulus to the reticulo-epithelial system leading to a temporary non-specific immune response, but further work on the subject is obviously desirable.

A practical reminder from this work is the possibility that the injection into animals of large numbers of organisms of a living 'avirulent' vaccine to test for the absence of pathogenicity may lead to wrong deductions being made as to the innocuity of the product.

## SUMMARY

An interference phenomenon was produced by the intraperitoneal injection of broth culture dilutions of *Pasteurella pestis* grown at 28° C. into small white rats.

At a critical level of approximately $10^6$ *Past. pestis* L37 marked interference was produced. Doses of $10^4$ or $10^7$ killed rats readily.

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Interference was produced by the addition of killed organisms, a cell-free vaccine or a live vaccine, to small lethal doses of L37.

Non-specific interference was produced by the intravenous injection of Indian ink prior to the intraperitoneal challenge with *Past. pestis* L37.

I should like to thank Dr D. W. Henderson for his criticisms.

REFERENCES

Boyd, J. S. K. (1951). *J. Path. Bact.* **63**, 445.
Evans, D. G. & Perkins, F. T. (1954). *Brit. J. exp. Path.* **35**, 603.
Green, R. G. (1945). *Amer. J. Hyg.* **41**, 7.
Henle, W. & Chambers, L. A. (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 713.
Pasteur, L. (1886). *Ann. Inst. Pasteur*, **1**, 1.
Vieuchange, J. (1940). *Bull. Acad. Méd., Paris*, **123**, 101.

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