STUDIES ON RESISTANCE TO BACTERIAL INFECTIONS IN ANIMALS INFECTED WITH RICKETTSIAE

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The phenomenon of interference between infectious agents, whereby infection with one organism protects animals against the effects of infection with another, has been recognized since 1929 when it was first reported by McKinney (1).

In 1935, it was again reported by Magrassi (2) and Hoskins (3) and since that time many articles on this subject have been published. Studies have been carried out on the interaction of viruses, both related (3–14) and unrelated (10, 15–20), between rickettsiae (21), on interference by viruses against bacterial infections (22), and by mycoses against rickettsial infections (23). Reviews of the studies on interference between animal viruses have been published by Henle (24) and Lennette (25).

The time and route of inoculation of the agents employed are reported to have considerable effect upon the phenomenon of interference in most of the systems studied. In general, that agent which is to exert the interfering action must be administered first (11, 13, 15, 18, 22) and the effective interval between administrations varies from 1 hour (13) to 15 days (18) or even 1 year (14). Occasionally, however, the effect may be observed when the two agents are administered simultaneously (3, 6, 16, 18, 21) or the interfering agent is injected last (6).

In some of the systems reported, the route of inoculation is of primary importance. Thus, in the studies by Dalldorf and his coworkers (22) on vaccinia virus and pertussis, interference was manifested when both agents were injected intracerebrally but not when they were instilled intranasally. Jordan and Duffy (12), on the other hand, found that St. Louis encephalitis virus interfered with Western equine encephalitis virus when both were instilled intranasally but not when they were injected intracerebrally. Jungeblut and Sanders (6) were able to protect monkeys against the simian strain of poliomyelitis by previous injection of the murine strain even when the two were administered by different routes.

The present paper is a report on the effect of such factors as those mentioned above on interference in a system in which infection with various species of rickettsiae protects animals against later infection with bacteria.

Materials and Methods

The animals employed were Swiss white mice from the colony maintained at the Rocky Mountain Laboratory and guinea pigs obtained from local breeders.

Rickettsia typhi was employed in the form of a pool of yolk sacs from infected embryonated eggs (26). This pool contained 1 ID_{50} for mice at the 10^9 dilution and the usual dosage was between 1×10^5 and 2×10^6 ID_{50} to mice and 5×10^6 mouse ID_{50} to guinea pigs.

The bacteria employed as challenge agents were *Pasteurella pestis* and *Pasteurella tularensis*. *P. pestis* strain 19 SP is highly infectious for both mice and guinea pigs when virulence is maintained by frequent guinea pig passages. Blood agar stock cultures were held at 5°C. For challenge, 48 hour, 37°C. growth on yeast beef agar was suspended in saline, centrifuged once, resuspended, and standardized by optical density (27). Challenge doses consisted of approximately 500 to 1,000 viable cells to mice and 5,000 to 10,000 cells to guinea pigs, checked by yeast beef agar plate counts.

The strains of *P. tularensis* employed were the highly virulent strain Schu, the moderately virulent strain 425-F4G, and the relatively avirulent strain Jap (27, 28). Stock cultures prepared at 6 week intervals from lyophilized ampules were maintained at 5°C. on cystine glucose blood agar. For challenge, suspensions of 24 hour, 37°C. cultures grown on the same medium, were prepared similarly to those of *P. pestis*, except that they were centrifuged twice. The number of cells of *P. tularensis* administered varied according to the virulence of the particular strain employed.

Unless otherwise specified, all injections to animals were administered by the intraperitoneal route. When administration was subcutaneous, *R. typhi* was always injected on the left shoulder and, when intramuscular, into the outer side of the left thigh. Subcutaneous or intramuscular injections of the challenge bacteria were administered into the same areas or into the opposite (right) side, according to the aims of the particular experiment.

Attempts to develop a strain of *P. pestis* resistant to the interfering action of *R. typhi* infection were made by injecting mice with the organisms in the usual manner: intraperitoneal injection of *R. typhi* followed 4 days later by similar injection of *P. pestis*. On the death of a mouse which had received both agents (failure of protection) the spleen of this animal was cultured for *P. pestis* and this culture was used to challenge the next group of mice.

RESULTS

In the system under investigation here, interference can be demonstrated in guinea pigs and mice and typical results in both species of animals are illustrated in Fig. 1.

Groups of 10 guinea pigs were treated as follows: one group was given 5×10^6 mouse ID_{50} of *R. typhi* intraperitoneally, another the same dose of rickettsiae and 4 days later 5,000 cells of *P. pestis* by the same route, and a third group was given *P. pestis* only.

The effect of previous infection with *R. typhi* on the time of survival after challenge with *P. pestis* was marked: all the animals which received *P. pestis* only succumbed before the first death occurred in the group which received both agents, and, in the later group, the percentage of deaths during the 14 day period of observation was reduced to 60 per cent. None of the rickettsial controls died. In groups of 50 mice treated similarly like results were obtained: 100 per cent of those infected with *P. pestis* only succumbed in 7 days and 56 per cent of those which also received rickettsiae in 10 days. Previous injection

of a suspension of yolk sacs from normal embryonated eggs had no effect upon the deaths from *P. pestis* infection.

Thus, the injection of *R. typhi* prior to administration of *P. pestis* had a marked influence on the courses of the bacterial disease in both animal species tested. Percentages of deaths were significantly decreased and the time of survival was often greatly prolonged among animals given both rickettsiae and bacteria as compared to those given bacteria only.

Effect of Length of Time between Administration of Agents.—The influence

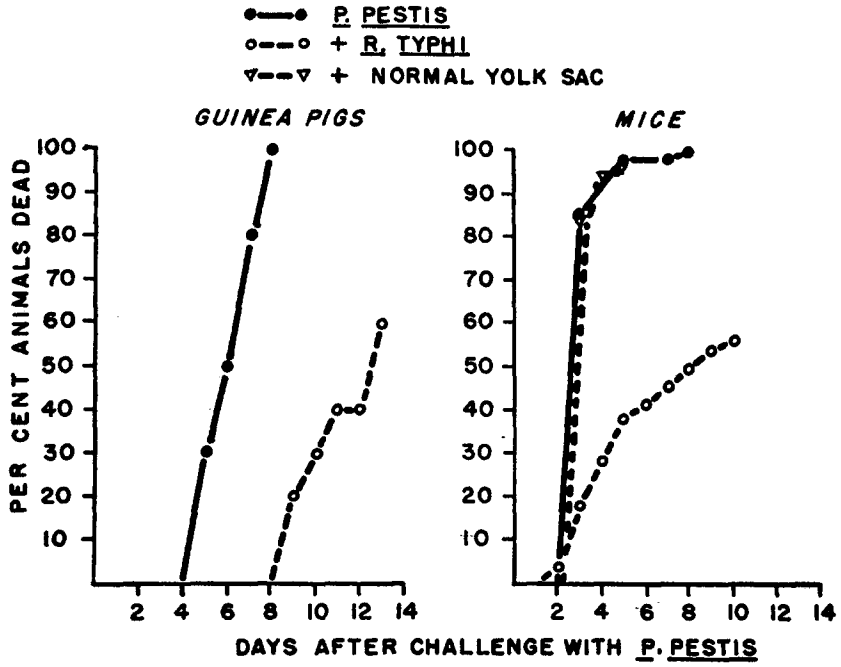

Fig. 1. Percentages of deaths in groups of 10 guinea pigs and 50 mice challenged with *P. pestis* 4 days after injection of *R. typhi*, as compared with those of animals injected with *P. pestis* only. One group of 50 mice received a suspension of normal yolk sac instead of the *R. typhi*.

of the period of elapsed time between administration of rickettsiae and challenge with bacteria in the present system was investigated.

Groups of 20 mice were injected at various times with 2×10^6 ID_{50} of *R. typhi* and later all were challenged simultaneously with 1×10^3 cells of *P. pestis*. The results are given in Table I.

When the challenge was performed immediately or 4 hours after injection of *R. typhi* the results were almost identical with those in the normal controls. Beginning with an interval of 16 hours between injections a degree of protec-

TABLE I

Effect of Interval between Injection of Rickettsiae and of P. pestis upon Death and Per Cent Mortality in Mice

Infected with		Interval between injections	Deaths on day after challenge													Deaths	
R. typhi	*P. pestis*		1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total*	Per cent
0	+	—			16	4											20/20	100
+	+	0			17	2	1										20/20	100
+	+	4 hrs.			12	6	2										20/20	100
+	+	16 "			6	1	2	3	1	2		4					19/20	95
+	+	2 days			2		1	5	2	1	1						12/20	60
+	+	3 "			1	2	1	1	6	2		2					15/20	75
+	+	4 "							1	2	1	1					5/20	25
+	0	—															0/20	0

* Numerator, No. animals dead; denominator, No. animals injected.

TABLE II

Effect of Route and Site of Administration of the Two Agents on Interference by R. typhi Infection with P. pestis Infection in Mice

Route of administration		Site*	Deaths on day after challenge											Death rate	
R. typhi	*P. pestis*		1	2	3	4	5	6	7	8	9	10	11	12	Total	Per cent
O	SC‡	—				4	5	2	3	1	3	1			19/20	95
SC		Same				2	1	2	1						6/20	30
SC		Different				1	2	1	3	2	2	2			13/20	65
IP		—					2	2	3	1		2		1	11/20	55
IM		—				3	4	3	3	2			2		17/20	85
O	IM	—				6	11	1	2						20/20	100
IM		Same			3	6	2	1		1				1	14/20	70
IM		Different				2	4	3	3	4	1		1		18/20	90
IP		—				1	5		4	2	3	1	1		17/20	85
SC		—			2	8	4	1		2	1		2		20/20	100
O	IP	—			3	8	2	3	1					1	18/20	90
IP		—		2		2			2			1			7/20	35
IM		—			3	7	3								13/20	65
SC		—			7	6	3								16/20	80
IP	O	—		1	1										2/20	10
IM		—													0/20	0
SC		—													0/20	0

* Same = both injections in same area of body. Different = injections on opposite sides of body.

‡ SC, subcutaneous; IM, intramuscular; IP, intraperitoneal.

tion was manifested by a prolongation of the time of survival in the mice which had received both agents. This protection became more marked as the interval increased, until, when 4 days had elapsed between the injections, only 25 per cent of the animals died. Similar experiments demonstrated that, when the interval was increased to 5 to 10 days, the degree of protection gradually declined, although the times of survival were somewhat longer than that of the controls.

The period of elapsed time between administration of rickettsiae and challenge with bacteria had, thus, an important influence on the degree of protection afforded by the rickettsial infection. The reaction which was responsible for this phenomenon was not instantaneous but required a certain length of time for its development. As a result of these findings, an interval of 4 days between administrations of the 2 agents was selected as the standard period for the remainder of the experiments.

Effect of Route and Site of Administration.—As has been noted, the phenomenon of interference is often most marked, or demonstrable only, when both infectious agents are administered by the same route and this has proved to be the case in the system under investigation here.

Groups of 20 mice were injected intraperitoneally, intramuscularly, or subcutaneously with 2×10^6 ID_{50} of *R. typhi* and 4 days later they and suitable normal controls were challenged with 1×10^3 cells of *P. pestis* by all 3 routes to all 3 groups. One-half of those which received both agents intramuscularly were challenged in the right thigh and the other half in the left thigh; the animals which received both agents subcutaneously were treated similarly. The results are shown in Table II.

There were mortality rates of 95 per cent among the mice receiving *P. pestis* subcutaneously, 65 per cent among those receiving both agents by this route but at different sites, and 30 per cent among those given both agents at the same site. In mice injected intramuscularly, comparable groups experienced death rates of 100, 90, and 70 per cent. Intraperitoneal administration of both agents caused the deaths of only 35 per cent of the animals, as compared to 90 per cent of those which received *P. pestis* only by this route. When the routes of administration were different, the rickettsial infection caused little or no interference with the infection by *P. pestis*.

Thus, the site of inoculation was of greater importance than the route of injection in the present system. Interference was most marked, or demonstrable only, when both injections were given into the same area and the phenomenon appeared to be largely the result of a local reaction.

Effect of the Number of Cells of P. pestis Administered in Challenge.—The number of cells of *P. pestis* used to challenge the animals had an influence on interference by rickettsial infection. The results of an experiment in which groups of 20 mice which had received *R. typhi* and groups of 20 normal mice were challenged with different decimal dilutions of *P. pestis* are illustrated in Fig. 2. When the inoculum consisted of 1×10^5 cells no significant difference

was found between the normal and the previously infected animals. As the numbers of *P. pestis* were decreased, the mortality rates of the mice which had

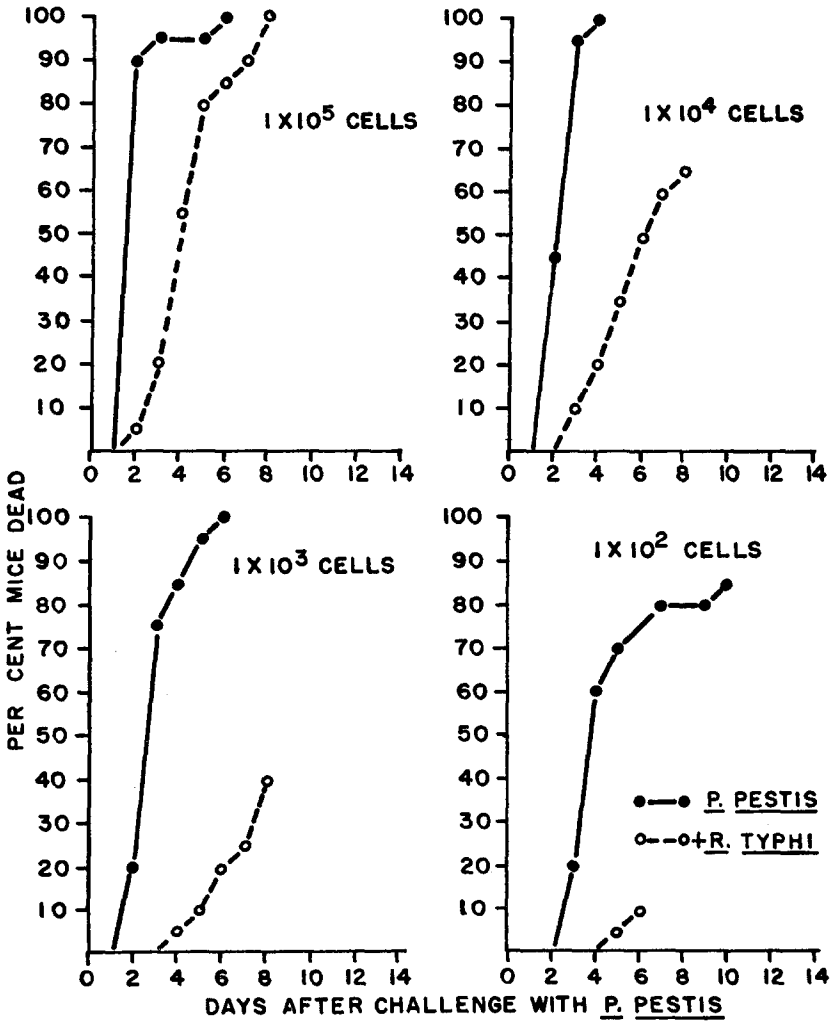

FIG. 2. Effect of the number of cells of *P. pestis* used to challenge groups of 20 mice with and without previous infection with *R. typhi*.

received rickettsiae also decreased and the time of survival in these animals was prolonged as compared to the controls. When the inoculum was reduced to 1×10^2 cells, only 10 per cent of the doubly infected animals died, compared to 83 per cent of the controls.

Thus, too large a number of bacteria will produce an infection so over-

whelming as to overcome any protective activity and too few will fail to kill a significant number of control animals.

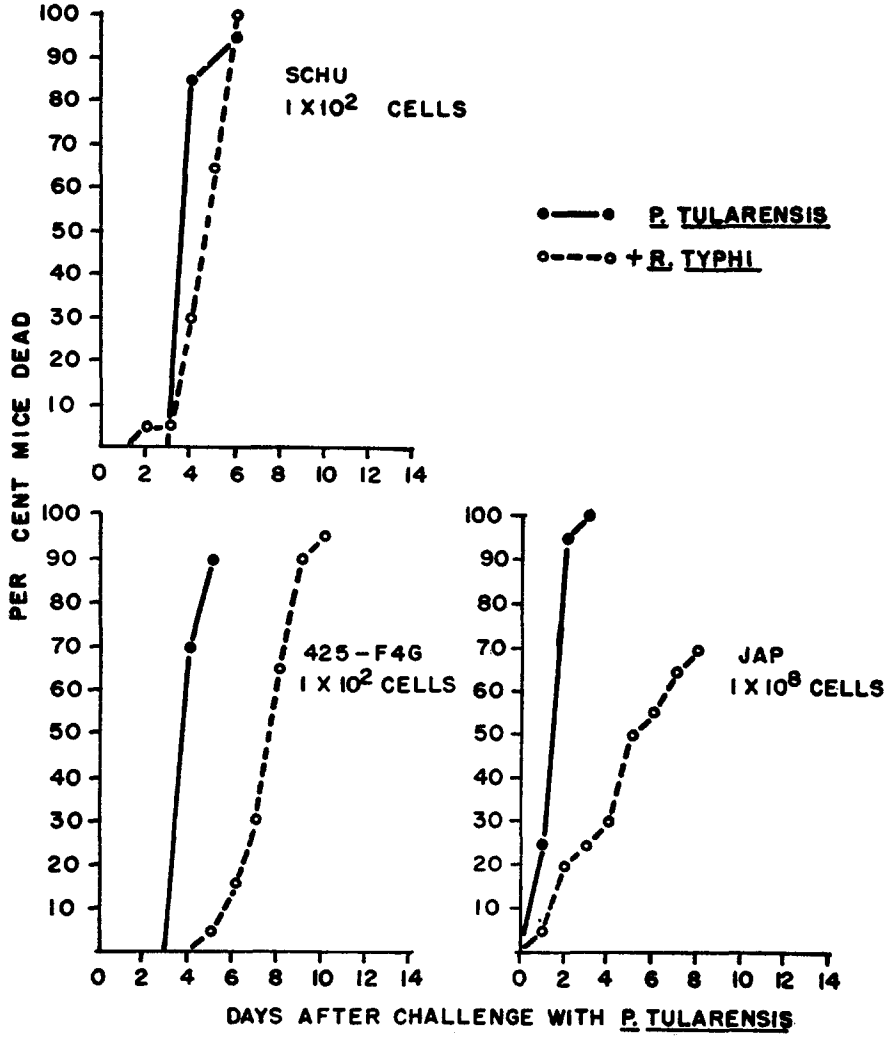

FIG. 3. Effect of the virulence of the strain of *P. tularensis* used to challenge groups of 20 mice with and without previous infection with *R. typhi*.

Effect of Virulence of Challenge Bacteria.—Fig. 3 illustrates that the virulence of the challenge organism has an effect on the phenomenon of interference similar to that of the numbers of organisms in the challenge dose. Because the virulence of *P. pestis* is very difficult to standardize, while that of

P. tularensis, under the conditions employed, is very stable (28), 3 strains of the latter species were used to investigate this subject in groups of 20 mice. With the highly virulent strain Schu there was no decrease in the per cent

TABLE III

Failure of P. pestis 19SP to Develop Resistance to Interfering Action of R. typhi

Passage No.	*R. typhi*	*P. pestis*	Deaths on day after challenge														Death total*	Rate
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
																		per cent
1	0	+			35	12	2										49/50	98
	+	+	1			1		3	3	1							9/40	22.5
2	0	+			14	26	5	2									47/50	94
	+	+		4	1	3	7	3	1		5						24/50	48
3	0	+			10	19	7	2	2		2	1	1				44/50	88
	+	+				1	1	1	2								5/50	10
4	0	+			3	7	11	5	3	1	1		1				32/50	64
	+	+			2		4	3									9/50	18
5	0	+			21	16	9			2		1					49/50	98
	+	+				4	3	5		3	2						17/50	34
6	0	+			15	14	12	4					1				46/50	92
	+	+		1	1	2	3		1	3		1					12/50	24
7	0	+			10	14	12	6	1	1					1		45/50	90
	+	+					1	2	3	7			2		1		16/50	32
8	0	+			13	17	10	2			1						43/50	86
	+	+			1	1	5	3	1	1		2					14/50	28
9	0	+			7	9	9	5	5	1		2					38/50	76
	+	+		1	1	1	1	2	3	1	1					1	12/50	24
10	0	+			13	21	12		1							1	48/50	96
	+	+		3	3	3	4	4	2	1	2						22/50	44

* Numerator, No. mice dead; Denominator, No. mice injected.

mortality nor increase in the time of survival in the animals infected with *R. typhi* as compared to those of the controls; with the moderately virulent strain 425-F4G, which has full virulence for mice, the mortality rate was not reduced but the time of survival was somewhat prolonged; while with the relatively avirulent strain Jap the deaths were reduced from 100 per cent in the controls

to 70 per cent in those receiving both agents and the time of survival was prolonged in the group which received rickettsiae.

Thus, a highly virulent strain is able to overcome the protection afforded by the rickettsial infection, while one of lower virulence will be susceptible to such activity.

Failure of P. pestis to Develop Resistance to the Interfering Action of R. typhi.—It was suggested to us by Dr. Victor Haas that the mechanism by which rickettsial infections interfere with bacterial infections might be of such nature that a strain of bacteria could develop resistance to it. In attempts to demonstrate the development of such resistance, 10 passages with the 19 SP strain of *P. pestis* were made through mice infected with *R. typhi*. In Table III it can be seen that these passages caused no significant decrease in the susceptibility of the strain to interference by infection with *R. typhi*. Even in the 10th passage a marked decrease in the deaths (from 96 to 44 per cent) and some prolongation in the time of survival occurred. Thus, it does not appear that the mechanism of interference in this system is such that bacterial challenge agent can develop resistance to it.

DISCUSSION

From the data reported here, the mechanism by which rickettsial infection interferes with infection by *P. pestis* or *P. tularensis* is not clear, but certain conclusions can be drawn.

The reaction responsible for this phenomenon is not instantaneous but requires a certain period of time, which appears in mice to be about 4 days, for the development of some function which enables the animal to suppress or overcome the challenge infection. More than 4 days after the administration of *R. typhi*, at least, the activity of this function appears to diminish, although some is detectable as long as 17 days later.

From observation of the animals it appears that the reaction in the doubly infected animal is more probably one of overcoming a more or less established infection than one of suppressing its development. Of mice, this is difficult to state, since animals which appear to be ill generally proceed to death in 1 to 3 days. On the other hand, guinea pigs which appeared acutely ill, with high temperatures and apparently extensive plague involvement, have survived and regained all appearances of healthy animals.

From the data reported here, the mechanism by which the bacterial infection is overcome appears to be largely a local phenomenon. The reaction to the rickettsiae of the involved local tissues, whether those of the peritoneal cavity, the muscles, or the subcutaneous areas, apparently aids the animal body in combatting the local challenging infection, perhaps partly by controlling and localizing it until sufficient immunity develops. That the reaction is not entirely confined to the local area was shown by smaller reductions in death

rates and the prolonged times of survival in animals which received the agents in different areas or by different routes (Table II).

Other species of rickettsiae (*R. prowazekii*, *R. rickettsii*, *R. akari*, and *Coxiella burnetii*) were also tested in the present system and all proved to be capable, to some extent at least, of interfering with infection by *P. pestis* in mice and guinea pigs. The results, however, were very irregular and are not given in detail here.

The function which overcomes the challenge infection is, apparently, not of such a nature that the challenge strain can develop resistance to it. This does not rule out the possibility that the interference may be due to some type of antibiotic activity on the part of the rickettsiae, but it would appear to be evidence against such an hypothesis.

SUMMARY

Data are presented, demonstrating that infection with *Rickettsia typhi* brings about a reduction of the death rates and a prolongation of the time of survival in animals subsequently challenged with *Pasteurella pestis* or *Pasteurella tularensis*.

This interference with bacterial infection by previous rickettsial infection does not appear immediately after injection of the rickettsiae; it begins to appear around 16 hours after this injection and becomes more marked during the first 96 hours; later it decreases.

The phenomenon is essentially a local tissue reaction with weaker systemic effects.

The phenomenon of interference can be overcome by challenge with too large a number or too virulent a strain of bacteria.

A strain of *P. pestis* subjected to 10 passages through mice infected with *Rickettsia typhi* failed to develop resistance to the interfering activity of the latter microbial species. This does not rule out, but might be evidence against, an assumption that the interfering action is due to antibiotic effects from the rickettsiae.

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