highly infective as determined by bioassay on primary cowpea leaves (*Vigna sinensis* Endl. var. Wilt Resistant), whereas those from uninoculated tobacco plants showed no virus activity.

*Comparison with tobacco-ringspot virus.* The tomato-ringspot and tobacco-ringspot viruses have similar host ranges (*4*), but the two viruses may be differentiated by cross-immunity tests and serologic reactions (*5*). It is apparent from the results reported here that the viruses may also be differentiated on the basis of particle size and shape. The tobacco-ringspot virus has been shown previously (*2*) to have an average particle diameter of 20.9 and 22 mμ, depending on the direction of measurement, and a polyhedral shape that approximates a sphere. On the other hand, the diameters of the tomato-ringspot virus were 43 and 13.5 mμ, with a polyhedral shape that resembles a flattened cylinder or pill.


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## Rickettsial-Interference Phenomenon: A New Protective Mechanism


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Previous studies from this laboratory have shown that under certain experimental conditions an interference phenomenon exists between certain rickettsial agents in guinea pigs (*1*). The data presented in this report (*2*) indicate that the rickettsial-interference phenomenon (RIP) is not due to competition between the rickettsiae for the same susceptible host cells. The isolation of a component from *Rickettsia rickettsii* is described that interferes with a challenge dose of a virulent strain of *Rickettsia rickettsii.*

*Quantitative studies on the number of susceptible cells infected during the rickettsial-interference phenomenon.* It has been shown that under certain conditions a low virulent T-type strain of *R. rickettsii* will protect guinea pigs against a highly virulent R-type strain of *R. rickettsii* (*1*). Further experiments were carried out to see whether this protection could be due to competition for the same susceptible host cells. Rickettsiae of a highly virulent R-type were purified from infected yolk sacs by the celite and albumin method of Bovarnick and Miller (*3*), and then counted under the electron microscope (*4*). The purified suspension was then inactivated by ultraviolet light. Various dilutions of this suspension were then injected intraperitoneally into guinea pigs, 15 animals being used for each dilution. After 3 hr, various numbers of living, highly virulent R-type strain of rickettsiae were injected intraperitoneally into the guinea pigs. This latter suspension was prepared and counted in the same way as the first suspension. Suitable controls were included in all experiments.

Three such experiments were carried out, all of them giving similar results. A typical experiment is shown in Table 1. It can be readily seen that the protection seems to be dependent on the ratio of interfering dose to infecting dose and not on the number of susceptible host cells.

It shoud be emphasized that there was little, if any, reactivation of the ultraviolet-treated organisms in the guinea pigs. This was shown by the fact that none of the guinea pigs receiving the treated rickettsiae showed any fever, and it takes only 100 living R-type strain organisms to produce 6 days of fever and even scrotal reactions in some of the animals, as is shown in Table 1. Furthermore, guinea pigs injected with $1 \times 10^{11}$ ultraviolet-inactivated rickettsiae were sacrificed at 2-day intervals for a period of 12 days, and their various organs were titrated in chick embryos (*4*). No viable rickettsiae could be demonstrated by this method, which is sensitive enough to detect at least 10 viable rickettsiae of the R-type strain (*4*). Injections of $1 \times 10^{6}$ ultraviolet-inactivated rickettsiae gave no protection even when the challenge dose was only 100 living virulent rickettsiae. If the living virulent organisms were given 3 hr before the inactivated rickettsiae, little, if any, protection was observed with any of the ratios shown in Table 1.

If the routes of inoculation of a low-virulent and of a high-virulent strain of *R. rickettsii* were changed,

Table 1. Effect of varying the number of rickettsiae on the interference phenomenon.

| No. of killed R-strain organisms injected | No. of living virulent R-strain organisms injected | Average days of fever | Scrotal reaction* | Fatality* |
|---|---|---|---|---|
| None | $1 \times 10^{2}$ | $6.4 \pm 0.92$ | 5/15 | 2/15 |
| $1 \times 10^{7}$ | $1 \times 10^{4}$ | $2.0 \pm 0.82$ | 2/15 | 0/15 |
| $1 \times 10^{7}$ | None | 0 | 0/15 | 0/15 |
| None | $1 \times 10^{4}$ | $6.1 \pm 1.1$ | 11/15 | 4/15 |
| $1 \times 10^{7}$ | $1 \times 10^{8}$ | $8.1 \pm 1.3$ | 9/15 | 11/15 |
| None | $1 \times 10^{8}$ | $7.6 \pm 1.2$ | 11/15 | 15/15 |
| $1 \times 10^{9}$ | $1 \times 10^{8}$ | $2.1 \pm 0.85$ | 2/15 | 0/15 |
| $1 \times 10^{9}$ | None | 0 | 0/15 | 0/15 |
| $1 \times 10^{9}$ | $1 \times 10^{10}$ | $8.1 \pm 1.3$ | 13/15 | 12/15 |
| None | $1 \times 10^{10}$ | $8.3 \pm 1.1$ | 14/15 | 15/15 |
| $1 \times 10^{11}$ | $1 \times 10^{10}$ | $2.3 \pm 0.81$ | 2/15 | 1/15 |
| $1 \times 10^{11}$ | None | 0 | 0/15 | 0/15 |

* The numerator indicates the number of animals showing scrotal reaction or dying of spotted fever.

Table 2. Effects of challenging guinea pigs by different routes.*

| Route of inoculation of low-virulent U-type strain (300,000 egg $LD_{50}$) † | Route of inoculation of high-virulent R-type strain (3000 egg $LD_{50}$) † | Average days of fever | Average height of fever | Scrotal reaction‡ |
|---|---|---|---|---|
| Intraperitoneal | Intraperitoneal | 1.9 ± 0.88 | 39.9 ± 0.11 | 1/15 |
| | Intraperitoneal | 8.1 ± 1.2 | 40.6 ± .12 | 12/15 |
| Intraperitoneal | Intramuscular | 3.4 ± 0.86 | 40.0 ± .13 | 0/15 |
| | Intramuscular | 5.0 ± 0.76 | 40.5 ± .11 | 2/15 |
| Intraperitoneal | Intracardial | 5.1 ± 0.98 | 40.4 ± .12 | 3/15 |
| | Intracardial | 7.6 ± 1.1 | 40.6 ± .09 | 5/15 |
| Intramuscular§ | Intramuscular | 1.9 ± 0.79 | 40.2 ± .14 | 0/15 |
| | Intramuscular | 4.8 ± 0.86 | 40.4 ± .12 | 2/15 |
| Intramuscular | Intraperitoneal | 1.8 ± 0.72 | 40.0 ± .12 | 1/15 |
| | Intraperitoneal | 7.3 ± 1.1 | 40.6 ± .07 | 10/15 |
| Intramuscular | Intracardial | 3.6 ± 0.92 | 40.3 ± .13 | 1/15 |
| | Intracardial | 8.1 ± 1.2 | 40.5 ± .11 | 5/15 |
| Intracardial | Intracardial | 4.4 ± 1.2 | 40.4 ± .13 | 3/15 |
| | Intracardial | 8.8 ± 1.3 | 40.6 ± .11 | 6/15 |
| Intracardial | Intraperitoneal | 1.0 ± 0.51 | 39.9 ± .14 | 1/15 |
| | Intraperitoneal | 7.5 ± 1.0 | 40.6 ± .14 | 11/15 |
| Intracardial | Intramuscular | 0.9 ± .63 | 40.0 ± .13 | 0/15 |
| | Intramuscular | 4.1 ± 1.2 | 40.4 ± .09 | 2/15 |

* All injections were in 0.2-ml amounts. The U-type strain by itself shows no detectable symptoms in guinea pigs.
† One egg $LD_{50}$ of either the U-type strain or the R-type strain is equal to about 20 to 100 organisms (*4*).
‡ The numerator indicates the number of animals developing scrotal reaction.
§ Intramuscular injections were all in the hind leg and in this case both injections were in the same hind leg.

the results shown in Table 2 were obtained. Doses of 300,000 egg $LD_{50}$ of a low-virulent U-type strain of *R. rickettsii* (*4*) and doses of 3000 egg $LD_{50}$ of a virulent R-type strain were used. The virulent strain was injected 4 hr after the low-virulent strain. This experiment was repeated four times with similar results.

*Isolation of a component from rickettsiae that will interfere with virulent rickettsiae.* An R-type strain of *R. rickettsii* was isolated from yolk sacs and purified by the celite and albumin procedure. It was then treated in a sonic vibrator for 30 min. After treatment, the suspension was centrifuged for 1 hr at 30,000 *g* and at 5°C. The supernatant fluid was collected; it was found to protect between 80 and 90 percent of the guinea pigs against a challenge of the virulent R-type strain. The R-type strain was given 3 hr after injection of the supernatant material. The supernatant fluid by itself caused no detectable symptoms in the guinea pigs. If a purified suspension was centrifuged for 1 hr at 30,000 *g* and at 5°C without being treated with sonic vibration, the supernatant fluid from such a suspension exhibited no interference effect and caused no symptoms.

These experiments, together with microscopic examination of such preparations and electron microscope pictures, have strongly indicated that the interfering effect of such preparations is not due to whole rickettsiae that might be present. About 30 percent of the rickettsial-interfering activity is recovered in these preparations. If this activity were due to whole rickettsiae, they should have been readily seen by the methods that were used. Concentrated preparations of the soluble antigen of *R. rickettsii* had no ability to interfere with the virulent rickettsiae.

The best preparations prepared by various fractionation procedures contained protein, lipid, carbohydrate, less than 0.1 percent of phosphorus, no pentosenucleic acid, and no desoxyribosenucleic acid.

*Discussion.* Any hypothesis to explain the rickettsial-interference phenomenon must account for these experimental facts: (i) Protection is dependent upon the ratio of interfering dose to the challenge dose but not upon the number of susceptible host cells. (ii) Mobilization and phagocytic activity of white cells, an inflammatory response, antibody formation, or the reversal of the protective effect by multiple infection of single cells by living virulent organisms do not play a major role in this phenomenon (*1, 5*). (iii) The challenge organisms are distributed in the same organs and tissues and to the same extent in both susceptible animals and animals where the interference phenomenon occurs, but there is much less multiplication of the challenge dose in the latter case (*1, 5*).

The relation of the RIP to the virus-interference phenomenon reported in experimentally inoculated animals is not clear. However, it should be pointed

out that the competitive "cell for cell" theory used to explain the virus-interference phenomenon has not been rigorously proved to occur in experimentally infected animals, since this explanation is based on bacterial-virus systems and the chick embryo-influenza system. Experiments are in progress to determine whether certain animal virus-interference systems are actually due to competition for susceptible host cells between two viruses.

*Note added in proof*: The RIP is highly specific under the experimental conditions shown in Table 1, nine bacterial species and nine viruses being tried as well as various other substances. All gave no interference. The RIP is also independent of the time that the challenge dose is given, provided that it is after the protective dose but not longer than about 10 days after the protective dose (*1*).

Recent results in animals, using a neurotropic virus system, have strongly indicated that in this case interference cannot be due to a saturation of susceptible cells by the protective dose. These results so far are very similar to those described here for the RIP.

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## Potassium and Sodium Balance in Mammalian Red Cells


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Estimations performed by flame spectrophotometry (*1*) of the red cell potassium and sodium content for nine mammals (man, baboon, rabbit, rat, horse, sheep, ox, cat, and dog) showed considerable species variation, whereas the chloride and calculated bicarbonate values were more constant (Table 1). The results confirm values by chemical methods for some species previously reported (*2, 3*). The individual red cell : plasma concentration ratios ranged from 1.5 to 32 for potassium and 0.1 to 0.9 for sodium; the red cell : plasma chloride and bicarbonate ratios were 1.2 to 1.6. The mechanism of distribution of potassium and sodium is, therefore, different from that of chloride or bicarbonate.

Blood specimens from the various species were collected in tubes containing heparin powder of low sodium content and were spun immediately under oil; the supernatant plasma, buffy coat, and superficial red cells were removed within 15 min of sampling to control cell-plasma ion and fluid exchange. The packed red cells were resuspended in an equal volume of:

1) One-percent NaCl-glucose-phosphate buffer, *p*H 7.6, at room temperature; a steady state with minimal electrolyte variation and normal glycolysis occurred for 6 to 12 hr (*1*).

2) Buffer with or without glucose and refrigerated at 2° to 7°C for 3 to 7 days; glycolysis was minimal; in accord with chemical concentration gradients, potassium diffused out and slightly more sodium in, with resultant cell swelling (Table 2*a*).

3) Cells from the refrigerated buffer were resuspended in isotonic saline-glucose-potassium, 5 milliequivalents per liter phosphate buffer solutions (*p*H, 6.8–8.0), in volume equivalent to that removed, and incubated at 37°C for 6 to 8 hr; restoration of glycolysis was associated with sodium extrusion slightly greater than potassium influx, with correction of cell volume and content (Tables 2*b* and 3).

Samples were removed periodically for potassium, sodium, and chloride estimations in the whole suspension and fluid medium, with calculation of the red cell values from determination of the red cell water and the packed cell volume corrected for trapped intercellular fluid (*1*). The hematocrit values were used to calculate water shifts in the system.

From chloride values and *p*H determinations of the fluid medium and red cell hemolysate, a Donnan ratio, $r$, $[Cl^-]_e/[Cl^-]_i = [H^+]_i/[H^+]_e$, calculated in log

Table 1. Ionic patterns of mammalian red cells; mean values. The notations [K], . . . denote milliequivalents per liter of red cell or plasma water.

| Species and No: estimated | Red cells | | | Plasma | | | Ratios | | |
|---|---|---|---|---|---|---|---|---|---|
| | [K] | [Na] | [Cl] | [K] | [Na] | [Cl] | $\frac{[K]_i}{[K]_e}$ | $\frac{[Na]_i}{[Na]_e}$ | $\frac{[Cl]_e}{[Cl]_i}$ |
| Man (120) | 136 | 19 | 78 | 5.0 | 155 | 112 | 27.4 | 0.16 | 1.44 |
| Baboon (56) | 145 | 24 | 78 | 4.7 | 157 | 115 | 30.8 | .15 | 1.48 |
| Rabbit (15) | 142 | 22 | 80 | 5.5 | 150 | 110 | 25.4 | .15 | 1.38 |
| Rat (36) | 135 | 28 | 82 | 5.9 | 152 | 118 | 23.0 | .18 | 1.44 |
| Horse (8) | 140 | 16 | 85 | 5.2 | 152 | 108 | 25.0 | .11 | 1.27 |
| Sheep (18) | 46 | 98 | 78 | 4.8 | 160 | 116 | 9.6 | .61 | 1.49 |
| Ox (28) | 35 | 104 | 85 | 5.1 | 150 | 109 | 6.8 | .69 | 1.28 |
| Cat (5) | 8 | 142 | 84 | 4.6 | 158 | 112 | 1.7 | .90 | 1.33 |
| Dog (28) | 10 | 135 | 87 | 4.8 | 153 | 112 | 2.1 | .88 | 1.44 |