creased cellulose digestion by rumen microorganisms *in vitro* (*8*).

References and Notes

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8. *Addendum.* Since this paper was submitted for publication Wise Burroughs *et al.* [*Science* **120**, 66 (9 July 1954)] have reported that the addition of diethylstilbestrol to the rations of fattening steers increased growth rate and feed efficiency.

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Particle Size and Shape of Purified Tomato-Ringspot Virus

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The particle size of the tomato-ringspot virus has been estimated at 50 mμ or less according to ultrafiltration techniques (*1*). The shape of such particles has not been described insofar as we are aware. Therefore, investigations were undertaken to determine the particle size with more precision and to ascertain its shape.

Purification of the virus. Tobacco plants of the Holmes' necrotic type with three or more fully developed leaves were inoculated with tomato-ringspot virus obtained from W. C. Price. Leaves showing typical symptoms after 18 days' incubation were frozen, minced, and extracted with dipotassium phosphate. The extract was then clarified by low-speed centrifugation. Initial concentration and purification were achieved by differential centrifugation in the preparative centrifuge, Spinco, Model L (*2*). The resultant pellets from both diseased and healthy tissues were suspended in 0.01*M* potassium phosphate buffer (*p*H 7) for each run. The pellet from the final centrifugation was resuspended in 0.1 ionic strength phosphate buffer (*p*H 6.5) for use in electrophoresis. Final purification was attained by electrophoresis for 312 min in the Tiselius apparatus.

Electron microscopy. Specimens for the electron microscope were prepared by the protein monolayer technique (*3*). This procedure involves the application of a suspension of virus particles in a dilute protein solution to a point on an aqueous surface. The protein spreads spontaneously and forms an insoluble monolayer in which the particles to be examined are uniformly distributed. The monolayer with its imbedded particles is then transferred to a celloidin-covered screen and shadowed with 8 A of uranium at a grazing angle of 16°. The magnification of an RCA-type EMU microscope was calibrated by a replica of a precision-ruled grating, consisting of 15,000 lines/in., manufactured by the Ford Company.

Particle size and shape. Preparations from the infected and virus-free tissue were examined with the electron microscope for differences in particle size and shape. Electron micrographs (Fig. 1) of preparations of an infective fraction from the electrophoresis apparatus indicate the presence of four- to six-sided particles having a cross section of 43 mμ when measured perpendicularly to the direction of the shadowing. The depth of the particle as obtained from shadow measurements was 13.5 mμ. Thus, the particles resemble flattened cylinders or pills. It is to be recognized that the measurement of shadows cast by individual particles is subject to uncertainty owing to local shadow angles, to the difficulty of making a precise determination of filament position, and to other factors. However, if it is assumed that this virus is not rigid and that the monolayer technique permits the distortion of the shape, then, from the dimensions given here, the volume may be equated to that of a sphere, in which case a diameter of 27 mμ is obtained.

Electron micrographs of preparations from virus-free plants contained many spheroidal particles approximately 14 mμ in diameter and much amorphous material. These particles were not observed in the electrophoretic fraction of extracts from virus-infected plants used for electron microscopy, but they were present in two of four other fractions. The polyhedral particles observed in infected tissue extracts as described in the preceding paragraph were not found in preparations from virus-free plants.

Infectivity. Aliquots of preparations from virus-infected plants containing the polyhedral particles were

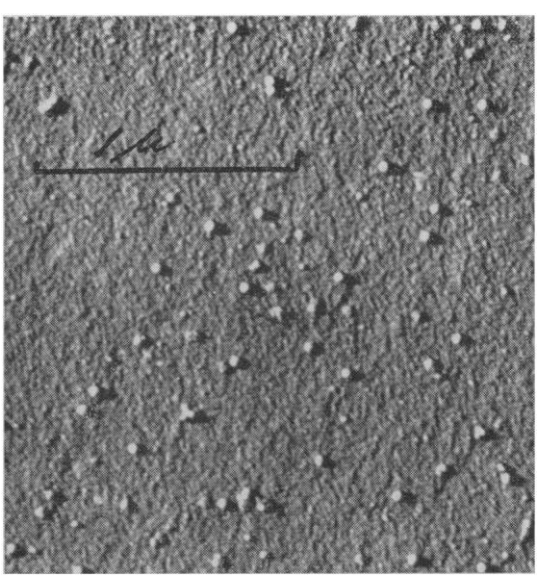

Fig. 1. Electron micrograph of particles from purified tomato-ringspot virus preparations (×33,000).

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 1×10^{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 1×10^{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	1×10^{2}	6.4 ± 0.92	5/15	2/15
1×10^{7}	1×10^{4}	2.0 ± 0.82	2/15	0/15
1×10^{7}	None	0	0/15	0/15
None	1×10^{4}	6.1 ± 1.1	11/15	4/15
1×10^{7}	1×10^{8}	8.1 ± 1.3	9/15	11/15
None	1×10^{8}	7.6 ± 1.2	11/15	15/15
1×10^{9}	1×10^{8}	2.1 ± 0.85	2/15	0/15
1×10^{9}	None	0	0/15	0/15
1×10^{9}	1×10^{10}	8.1 ± 1.3	13/15	12/15
None	1×10^{10}	8.3 ± 1.1	14/15	15/15
1×10^{11}	1×10^{10}	2.3 ± 0.81	2/15	1/15
1×10^{11}	None	0	0/15	0/15

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