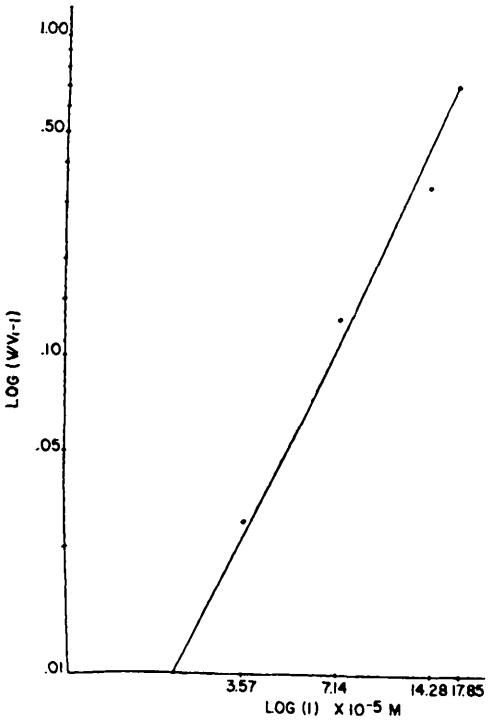

FIG. 4. Double log plot showing slope of line equal to 2. Method described by Ebersole *et al.* (12).

tectable *in vitro*, have little or nothing to do with their physiological effects when injected. The androgens have not been as thoroughly studied as the estrogens in this regard. The inhibitory property of testosterone-17β diethyl aminoethyl carbonate might reside, for the most part, in the C-17 substituted grouping, as has been previously suggested(5). In spite of this, the compound was used in the present study because of the technical advantage of its water-solubility.

Summary. It has been demonstrated that the *in vitro* inhibition of rat liver succinoxidase activity by testosterone-17β diethyl aminoethyl carbonate is non-competitive, with 2 molecules of inhibitor apparently interacting with one molecule of enzyme.

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Interference Phenomenon in Animal Infections with Rickettsiae of Rocky Mountain Spotted Fever.* (20060)

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The interference phenomenon has been

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demonstrated for many animal, plant, and bacterial viruses. Although they present endless variety and detail, the general characteristics of these phenomena are well established. While there have been no reports of interference between rickettsiae, it seemed possible that these agents might also be subject to infection interference, since rickettsiae are also intracellular parasites. In the course of studies on the epidemiology of Rocky Mountain spotted fever, the experiments which are here reported were undertaken to test the validity of this hypothesis.

Materials and methods. The two strains of *R. rickettsii* employed in these experiments have been previously described(1). Strain T is of low virulence, rarely causing scrotal reactions and is not fatal for guinea pigs. The R strain, on the other hand, causes a marked scrotal reaction resulting in necrosis, and kills some of the inoculated guinea pigs. Strains R and T are similar in that both possess toxin, a hemolytic factor, and are practically immunologically identical(1). Material for inoculation was obtained by egg passage, using chick embryo yolk sacs. All egg titrations to determine the LD_{50} of the various suspensions were carried out in 5-day-old chick embryos. 0.5 ml of the various suspensions was inoculated into the yolk sac and incubated at 35°C. Eggs dying within 48 hours were discarded. The remaining eggs were examined daily for 12 days. Ten to 12 eggs were used per dilution. All dilutions were carried out in Snyder's solution(2). The strains of other rickettsiae employed were those being carried at the Rocky Mountain Laboratory (U.S.P.H.S.), Hamilton, Montana. The Nine-Mile Q fever strain, a Malayan mite strain of scrub typhus, the Brienl strain of epidemic typhus, a strain of Boutonneuse fever, and the Wilmington strain of endemic typhus were employed in the various experiments. Egg material was used for inoculation for all of the above strains except in the case of scrub typhus, where mouse spleen was used. All guinea pigs used were healthy male animals weighing between 350-450 g. Of the wild animals, the Columbian ground squirrels, field mice (Microtus), and cottontail rabbits were trapped in the Bitterroot Valley, Montana. None of the animals employed showed any complement-fixing antibodies against any of the spotted fever strains used in these studies. The *D. andersoni* nymphs used in all experiments had been infected as larvae and nymphs with either the T or the R strain by feeding on infected guinea pigs. After the female laid eggs, the resulting larvae were infected with the appropriate strain and allowed to molt to nymphs. The above procedure was repeated once more.

TABLE I. Interference between Infection with a Strain of Low and One of High Virulence of *R. rickettsii*, as Demonstrated by Injection into 5 Series of 10 Guinea Pigs Each.

Strain injected	Fatality	Avg days of fever	4+ scrotal reaction
T	* 0/50	5.1 ± .92	† 0/50
R	14/50	8.1 ±1.2	45/50
T + R	0/50	5.3 ± .82	6/50

* Numerator shows No. of animals dying from spotted fever.

† Numerator shows No. developing severe scrotal reactions. Four animals inj. with T strain followed by R strain developed a slight scrotal reaction leading to swelling and redness which disappeared after a few days.

Results. Two series of experiments were performed. The first series of experiments shown in Table I were carried out as follows: 3×10^5 LD_{50} (determined by egg titration) of the weakly virulent T strain was injected intraperitoneally into 10 male guinea pigs. Four hours later, 10^4 LD_{50} (determined by egg titration) of the virulent R strain was injected intraperitoneally into the above animals. Ten control animals were injected with 3×10^5 LD_{50} of the T strain, and 10 guinea pigs with 10^4 LD_{50} of the R strain. The animals were observed for 2 weeks. A temperature of 39.8°C or over was considered as fever. The results are cumulative data from 5 experiments, all giving the same qualitative and approximately the same quantitative results. 4+ scrotal reactions indicate severe reaction leading to hemorrhage and necrosis. From Table I it is obvious that a weakly virulent strain of *R. rickettsii* protected animals against a virulent strain of the disease. Other experiments showed that under conditions similar to those shown in Table I, strain T (a) protected animals when both T and R were given simultaneously, or when R was

injected anywhere up to 14 days after the T strain (cf. footnote‖), (b) protected about 40% of the guinea pigs when only 3×10^4 LD_{50} of strain T was injected, (c) interfered with the R strain when inactivated by ultraviolet irradiation but not by heat, and (d) protected animals against boutonneuse fever.

In other experiments involving quantitative centrifugation and highly purified rickettsiae (1) the results indicated that the protective property is a part of the rickettsiae and not some other substance present in the infected yolk sacs.

Suspensions of rickettsiae causing Q fever, scrub typhus, epidemic typhus, and endemic typhus, which are immunologically unrelated to *R. rickettsii,* were substituted for Rocky Mountain spotted fever strain T. Under the conditions shown in Table I, suspensions of these immunologically distinct rickettsial strains also protected about the same percentage of animals against injections of the virulent R strain. The animals did develop the typical symptoms caused by the rickettsiae used to protect against spotted fever. Not only did these rickettsiae protect animals against the symptoms of Rocky Mountain spotted fever, but in guinea pigs inoculated with *C. burneti* (Q fever) followed 4 hours later by an injection of *R. rickettsii,* there was 1000 times less the maximum number of Rocky Mountain spotted fever organisms in the testis-tunica than was found in the control animals, which received an injection of strain R alone. A typical experiment is shown in Table II. Twelve male guinea pigs were injected intraperitoneally with 1×10^6 LD_{50} (determined by egg titration) of *C. burneti.* Four hours later they were injected intraperitoneally with 1×10^4 LD_{50} of strain R of *R. rickettsii.* Twelve control guinea pigs were injected intraperitoneally with 1×10^4 LD_{50} of strain R. At 8, 72, 120, and 230 hours following inoculation of strain R, the testes-tunicas were taken from 2 pigs from each group and combined, and 10% suspensions prepared in Snyder's solution(2). Each suspension was titered in guinea pigs which had recovered from an infection of *C. burneti.* These animals had been injected with 1×10^5 LD_{50} of *C. burneti* 2 months previously. All these

TABLE II. Multiplication of *R. rickettsii* in Testes-Tunicas of Guinea Pigs Injected 4 Hours Previously with *C. burneti.*

Time after *R. rickettsii* inj. (hr)	Min. infectious doses (ID_{50}) in testis-tunica suspensions	
	Q + R	R
8	0	0
72	10	1000
120	1	10000
230	0	100

guinea pigs showed complement-fixing antibodies of over 1:512. Five of these animals were used per dilution to titrate each testis-tunica suspension. The control series were also titrated in the Q fever immune animals. Control experiments showed that the testis-tunica suspensions from the animals injected with *C. burneti* and *R. rickettsii* contained no demonstrable inhibitors, which would prevent the suspensions from showing higher spotted fever titers if larger numbers of strain R of *R. rickettsii* had been present.

The second series of experiments shown in Table III were carried out as follows: 100 *D. andersoni* nymphs infected with the low virulent strain T and 2 *D. andersoni* nymphs infected with the highly virulent strain R were put on each of 3 Columbian ground squirrels. After 3 days at which time 80% of the nymphs infected with the T strain and both nymphs infected with the R strain had been feeding, 100 nymphs which had been raised through many generations in the laboratory and were free of spotted fever, were put on each of the 2 squirrels. The uninfected nymphs were allowed to engorge and molt to adults. After one month at room temperature, 30 nymphs from each squirrel were put on each of 2 guinea pigs. The nymphs were allowed to feed for 6 days and the animals then observed for 3 weeks. In control experiments, the lowly virulent and highly virulent infected samples (Table III, 1 and 2) were set up exactly as described above except that sample 1 had no highly virulent infected ticks and sample 2 no low virulent infected ticks. The results are the cumulative data from 4 experiments, all experiments giving the same results. It can be readily seen that uninfected ticks feeding on Columbian ground squirrels harboring a large number of *D. andersoni* in-

TABLE III. Interference between Infections with a Strain of Low and One of High Virulence of *R. rickettsii* as Demonstrated by Feeding *D. andersoni* Ticks on Ground Squirrels.

Normal ticks fed on squirrels infected by exposure to		Reactions of guinea pigs on which normal ticks fed after feeding on squirrels		
		Avg days of fever	Scrotal reaction	Fatality
Low virulent infected ticks	(1)	5.1 ± .93	* 0/20	†0/20
Highly	(2)	8.2 ±1.4	17/21	8/21
Low and highly	(3)	4.6 ± .81	0/19	0/19

* Numerator shows No. of animals showing scrotal reaction. Four animals in group (1) showed no symptoms, 3 animals in group (2) showed no symptoms, and 5 animals of group (3) showed no symptoms. Fever equals any temperature over 39.8°C. All scrotal reactions were severe resulting in necrosis in group (2).

† Numerator shows No. of animals dying of spotted fever.

fected with a low virulent strain and a small number of *D. andersoni* infected with a highly virulent strain of *R. rickettsii*, transmit only a low virulent strain to animals. It is not possible to say from these experiments whether interference between the high and low virulent strains occurs in the ticks. All tests carried out so far indicate that such ticks contain only the low virulent strain. Larvae coming from female of such ticks have been found to have the same strain as the parent. Experiments similar to the above were carried out with field mice (Microtus) and cottontail rabbits with similar results. In other experiments, in which ticks harboring highly virulent strains predominated, "normal" ticks became infected only with the highly virulent strain. Larvae coming from females of such ticks have been found to have the same strain as the parent.

Discussion. The current hypothesis is that interference between viruses is due to competition for susceptible cells. This idea is based mainly on the work carried out with bacteriophage and the influenza-chick embryo system.

In the experiments described in this paper, in which various strains of rickettsiae protect guinea pigs against virulent spotted fever, there are many similarities to the interference phenomena reported for viruses. However, two lines of evidence indicate that the protection against virulent spotted fever may not be due to competition for susceptible cells. In the first place, only 10^7 to 10^8 rickettsiae‡ per animal were injected to establish protection. Taking into account that spotted fever is a rather generalized infection it does not seem likely that anywhere near all the susceptible cells are invaded. Indeed, direct evidence supporting this contention has recently been found. Secondly, 15§ days after the animals are injected with Q fever rickettsiae, which are immunologically distinct from *R. rickettsii*, the animals are no longer protected against an injection of virulent spotted fever,‖ although at this time all the organs show many more cells infected with Q fever rickettsiae than were present in the initial 4 hour post-inoculation time, during which time the animals were protected against an injection of virulent *R. rickettsii*. Thus, while there are similarities between the experiments described in this paper and those involving virus interference, future experi-

‡ The rickettsiae were counted by the electron microscope method described previously(1).

§ Animals injected with Q fever are resistant to the R strain to about 10 days post-inoculation. That is to say, animals injected with Q fever are not resistant to an injection of spotted fever given about 2 weeks later. At no time during the course of infection in Q fever infected animals, including first to thirtieth day post-inoculation period, does sera of such animals give any antibody reaction against R strain as measured by complement fixation activity, neutralization of spotted fever toxin in mice.

‖ This does not hold for the low and highly virulent strains of spotted fever since immunologically they are practically identical, and by 15 days antibodies begin to appear in blood of the animal. From this time on, therefore, protection given by the low virulent strain against virulent strain may be due entirely to immune response.

ments must decide whether the "interference phenomenon" observed in the two classes of infectious agents is due to the same mechanism.

The "interference phenomenon" reported in this paper involving *D. andersoni* and small wild animals may have important implications in explaining the epidemiology of Rocky Mountain spotted fever. It may be involved in the explanation of the contention that in certain limited localities strains having high and low virulence persist year after year(3). Since a similar situation is found in scrub typhus(4), it is possible that such an "interference phenomenon" is important in the epidemiology of this rickettsial disease. However, much more work is necessary before final conclusions can be drawn, since many factors would be involved in determining the importance of such a phenomenon in nature.

It should be mentioned that with certain strains of lower virulence than the T strain, it has not been possible to infect cottontail rabbits, Columbian ground squirrels, field mice, or chipmunks, so that such animals will infect *D. andersoni*. These animals are among the main animal reservoirs for larvae and nymphs of *D. andersoni*. Multiplication of the rickettsiae of these strains of very low virulence could also not be demonstrated by repeated egg titrations of the organs and blood of the above animals. On the other hand, these strains grow about as well in *D. andersoni* and chick embryos as the virulent strains. It is possible that they are maintained by hereditary transmission. These low virulent strains, however, will still protect animals against highly virulent strains under the conditions described in Table I. The elucidation of the mechanism by which these strains are maintained in nature is of importance in understanding the epidemiology of not only Rocky Mountain spotted fever, but perhaps also of other rickettsial diseases.

Summary. 1. Eighty to 90% of the guinea pigs infected intraperitoneally with a strain of low virulence of *R. rickettsii* were protected against a simultaneous injection of a highly virulent strain of spotted fever, or boutonneuse fever, provided the low virulent strain was given in about 10 to 30 times the concentration of the virulent strain. 2. Infection with rickettsiae of Q fever, scrub typhus, endemic typhus, and epidemic typhus protected guinea pigs against a virulent strain of spotted fever under the above conditions. 3. Columbian ground squirrels, field mice (Microtus), and cottontail rabbits were infected by exposure to many *D. andersoni* containing a low virulent strain of Rocky Mountain spotted fever and a few *D. andersoni* containing a highly virulent strain. Uninfected ticks fed upon these animals were found to contain only the low virulent strain.

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Role of Thioctic Acid in the Transfer of Acyl Groups.* (20061)

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Preliminary data(1) have suggested that in the oxidation of pyruvate thioctic acid has no role in the transfer of electrons over DPN,†

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† The following abbreviations are used: Co A, coenzyme A; $DPN_{ox.}$ and $DPN_{red.}$, oxidized and reduced diphosphopyridine nucleotide; ATP, adenosine triphosphate.