TABLE 9.—*Number of children of each age with each specified vision in each eye—9,245 native white children of South Carolina, Maryland, Delaware, and New York State.*

Vision.	All ages.	Age nearest birthday.										
		6	7	8	9	10	11	12	13	14	15	16
BOTH SEXES.												
Right eye, total	9,245	393	933	1,121	1,225	1,230	1,108	1,050	906	686	390	203
Normal	6,370	253	595	735	829	849	746	767	671	496	281	148
6/10, 7/10, or 8/10	2,182	115	263	317	327	273	284	199	160	135	73	36
4/10 or 5/10	437	18	52	52	47	68	53	45	43	29	18	6
1/10, 2/10, or 3/10	256	7	17	17	22	40	25	39	32	26	18	13
Left eye, total	9,245	393	933	1,121	1,225	1,230	1,108	1,050	906	686	390	203
Normal	6,378	259	611	747	818	843	758	759	646	494	291	152
6/10, 7/10, or 8/10	2,171	104	260	289	330	287	267	217	170	145	68	34
4/10 or 5/10	441	24	47	66	56	57	48	41	46	32	15	9
1/10, 2/10, or 3/10	255	6	15	19	21	43	35	33	44	15	16	8
BOYS.												
Right eye, total	4,774	205	492	590	631	683	568	533	444	339	193	96
Normal	3,389	131	324	392	430	492	398	406	336	265	139	76
6/10, 7/10, or 8/10	1,040	61	131	167	160	130	128	93	75	47	36	12
4/10 or 5/10	216	8	29	24	29	38	27	17	19	15	8	2
1/10, 2/10, or 3/10	129	5	8	7	12	23	15	17	14	12	10	6
Left eye, total	4,774	205	492	590	631	683	568	533	444	339	193	96
Normal	3,388	133	328	393	433	483	399	412	319	264	142	77
6/10, 7/10, or 8/10	1,031	57	130	148	155	139	131	88	82	53	35	13
4/10 or 5/10	224	12	24	36	34	34	18	20	21	15	7	3
1/10, 2/10, or 3/10	131	3	10	8	9	27	20	13	22	7	9	3
GIRLS.												
Right eye, total	4,471	188	441	531	594	547	540	517	462	347	197	107
Normal	2,981	122	271	343	399	357	348	361	335	231	142	72
6/10, 7/10, or 8/10	1,142	54	132	150	167	143	156	106	85	88	37	24
4/10 or 5/10	221	10	29	28	18	30	26	28	24	14	10	4
1/10, 2/10, or 3/10	127	2	9	10	10	17	10	22	18	14	8	7
Left eye, total	4,471	188	441	531	594	547	540	517	462	347	197	107
Normal	2,990	126	283	349	385	360	359	347	327	230	149	75
6/10, 7/10, or 8/10	1,140	47	130	141	175	148	136	129	88	92	33	21
4/10 or 5/10	217	12	23	30	22	23	30	21	25	17	8	6
1/10, 2/10, or 3/10	124	3	5	11	12	16	15	20	22	8	7	5

ROCKY MOUNTAIN SPOTTED FEVER: EXPERIMENTAL STUDIES ON TICK VIRUS.

By R. R. SPENCER, Surgeon, and R. R. PARKER, Special Expert, Hygienic Laboratory, United States Public Health Service.

The virus of Rocky Mountain spotted fever may be studied as it occurs in the tissues of susceptible mammals (tissue virus) or in the intermediate hosts, *Dermacentor andersoni* Stiles and *Haemaphysalis leporis-palustris* Packard[1] (tick virus). Observations during the

[1] Parker, R. R.: Transmission of Rocky Mountain Spotted Fever by the Rabbit Tick, Haemaphysalis leporis-palustris Packard. Am. Jour. Trop. Med., Vol. III, No. 1, January, 1923.

past two years have indicated that tick virus possesses interesting and perhaps significant phases in its development not observed in tissue virus. In a previous paper [2] we described a nonvirulent immunity-producing phase of the tick virus in unfed infected ticks, and a reactivation of such virus to a virulent infectious stage following the ingestion of fresh blood by the ticks. These preliminary studies were made on infected ticks collected in nature. For further and more detailed observations, ticks have been infected under controlled conditions. To this end, the progeny of single noninfected females have been used as units designated by lot numbers and have been infected by permitting them to engorge during either the larval or nymphal stage upon a rodent inoculated with spotted fever virus. In this way many infected ticks of identical history have been secured. Furthermore, this method of infecting ticks is comparable in large measure to that taking place in nature, since the immature stages [3] feed on wild rodents susceptible to spotted fever.

On the other hand, infection is seldom acquired by adult ticks, the majority of which feed on nonsusceptible large mammals, wild and domestic.

In previous experimental work the importance of infecting ticks during one of the immature stages has not been emphasized. While adult ticks are more readily obtained and more easily handled and controlled than the smaller nymphs and larvæ, nevertheless, infected at the adult stage, they often fail to transmit the fever when permitted to feed again upon a healthy animal, although the injection of their viscera soon after feeding is usually successful.[4] However, adult females receiving the infection and permitted to come to full engorge-

[2] Spencer, R. R., and Parker, R. R.: Rocky Mountain Spotted Fever: Infectivity of Fasting and Recently Fed Ticks. Pub. Health Rep., Vol. 38, No. 8, Feb. 23, 1923, pp. 333-339. (Reprint No. 894.)

[3] *D. andersoni*, like many other external parasites, undergoes an interesting and complicated life cycle. The adult female, after engorging to many times its normal size, drops from its host and crawls to a sheltered place. Before leaving the host the female is impregnated by the male, which feeds only a short time before seeking its mate. The female remains quiescent a week or more, depending on the temperature, and then begins the deposition of eggs—from 2,000 to 7,000 in number. This sometimes takes a month or even longer. These hatch to seed ticks or larvæ, which are not more than one thirty-second part of an inch in their longest diameter. These six-legged larvæ feed on rodents, such as ground squirrels, chipmunks, field mice, rabbits, etc.; 50 or more may be found on one small host. After feeding from two to four days and attaining the size of a millet seed, they drop to the ground, pass through a dormant stage, and shed the outer skin, emerging as eight-legged nymphs which are sexually undifferentiated. They do not become active, however, until the following spring. Like the larvæ, they feed on rodents and engorge in from 3 to 10 days, finally reaching a size slightly smaller than buckshot. The engorged nymphs then molt to the adult ticks—males and females—which pass the winter in a dormant condition. The adults attach themselves only to large animals, including man, and are seldom found on animals smaller than a jack rabbit. The larval and nymphal ticks, on the other hand, have never been found on any but small animals, though occasionally nymphs have been removed from children. Although under normal conditions the cycle from egg to adult is completed in two years, it frequently happens that the ticks do not secure a host during the season in which they become active. This causes a high mortality among the larvæ and nymphs. The adults are able to survive for two, three, and occasionally four years without feeding. In this way the life cycle may be considerably lengthened. In the laboratory, however, they are often forced by artificial means to complete the cycle in three months.

The life cycle of *Haemaphysalis leporis-palustris* (rabbit tick) has not been completely worked out for this locality (Hamilton, Mont.).

[4] Spencer, R. R., and Parker, R. R.: Loc. cit.

ment may transmit it through the eggs to the next generation. The infection may then be recovered in animals by (*a*) injection of deposited eggs—a single egg (0.0006 gm.) has been found infective—(*b*) feeding the resultant larvæ or nymphs, (*c*) injection of the larval or nymphal contents. The last test should preferably be made soon after molting and before hibernation of the larvæ or nymphs.

On the other hand, adults which have been infected during one of the immature stages of the life cycle, if tested after passing through the winter, readily infect animals by feeding, whereas injection of tick contents does not infect unless the ticks are first incubated or fed. Further, the virus in fed adults infected in either of the early stages has been found to be more highly fatal and more concentrated than either tissue virus or tick virus at other stages. Recent tests, not included in this paper, indicate considerable concentration of virus in engorged nymphs which have been infected as larvæ, and in engorged larvæ which have been infected in the previous generation. In addition, the killed virus of such adult ticks possesses an immunizing quality never encountered in blood virus.

Before giving our data in support of these statements certain general considerations relative to the underlying conditions in nature and upon which the maintenance of Rocky Mountain spotted fever rests should be briefly reviewed. Chief among such considerations are—

1. The disease is maintained in rodents and ticks; human cases are secondary and accidental.

2. The disease exists in definite foci, and the virulence of the infection may vary decidedly even in adjacent areas.

3. A large number of rodent species are susceptible, but there is no evidence that the infection is highly fatal among them.

4. The complete life cycle of the tick includes four stages, and the infection may pass from stage to stage and from one generation to the next.

5. A disintegration of tissue (histolysis) takes place during the premolting period of larvæ and nymphs.

6. The tick ingests mammalian blood three times during the cycle—twice from small rodents (most of them susceptible to spotted fever) as larvæ and nymphs and once from large animals (all immune as far as known except that some adults feed on jack rabbit, snowshoe rabbits, and porcupines, of which animals at least the first two are susceptible to spotted fever) as adults.

7. The virus passes through the egg and larval stages of the tick in one summer, but in the unfed nymphs and adults it has been compelled to adapt itself to the hibernation (also æstivation in the adult) which these stages undergo.

14469°—24†——2

8. The infective agent experiences a sudden change in environment when it passes from mammalian blood to that of the insect host, and vice versa.

9. The mammalian host provides a far more regular and unchanging environment as regards temperature for the virus than the cold-blooded tick.

1. DEVELOPMENTAL PHASES OF TICK VIRUS.

In Table 1 it may be seen that infected adult ticks, infected as larvæ, lot 2351–B [5] recently molted and not subjected to cold, produced spotted fever when injected intraperitoneally into guinea pigs. Ticks from the same lot after 39 days, and again after 112 days, in the ice box did not produce fever upon injection. However, 4 out of 8 ticks so tested immunized the guinea pigs against 1 c. c. of blood virus given 10 days after the injection. Yet simultaneous tests with ticks from the same lot incubated at 37° C. for 24 hours after removal from the ice box produced spotted fever in 6 out of 8 guinea pigs.

TABLE 1.—*Studies of tick virus in adults of lot No. 2351–B–(2A)—Intraperitoneal injection of tick viscera into guinea pigs, contents of 1 tick into each pig. All pigs surviving tick inoculation 10 days or more were given 1 c. c. of blood virus.*

Test No.	Date.	Condition or preparation of tick.	Result of guinea pig inoculation.
1	Sept. 12, 1923	Recently molted, kept at room temperature	Typical spotted fever.
2	----do	----do	Do.
3	----do	----do	Do.
4	Oct. 31, 1923	Ice box 39 days, Sept. 12 to Oct. 31	No fever.
5	----do	----do	Do.
6	----do	----do	Do.
7	----do	----do	No fever; later immune.
8	Nov. 1, 1923	Ice box 39 days; 37° C. for 24 hours	Typical spotted fever.
9	----do	----do	Do.
10	----do	----do	Do.
11	----do	----do	No fever; later immune.
12	Jan. 2, 1924	Ice box 112 days, Sept. 12, 1923, to Jan. 2, 1924	No fever.
13	----do	----do	No fever; later immune.
14	----do	----do	Do.
15	----do	----do	Do.
16	Jan. 3, 1924	Ice box 112 days; 37° C. for 24 hours	No fever.
17	----do	----do	Typical spotted fever.
18	----do	----do	Do.
19	----do	----do	Do.

[5] History of lot 2351–B—

Apr. 11, 1923: One fully engorged female tick secured from a horse west of Hamilton, Mont.

May 5, 1923: Eggs deposited by female hatched to larvæ.

July 1, 1923: Larvæ placed on a Belgian rabbit which had previously been inoculated with 1 c. c. of *guinea pig virus*.

July 9, 1923: Twenty-five fully engorged larvæ injected into guinea pig No. 3986. Typical spotted fever developed.

Aug. 8, 1923: Engorged larvæ had now molted to flat nymphs, and the latter were placed on a normal Belgian rabbit.

Aug. 15, 1923: Five engorged nymphs injected into guinea pig No. 4637. Animal developed typical spotted fever.

Sept. 8, 1923: Engorged nymphs had all molted to adults, some of which were forwarded to the Hygienic Laboratory at Washington and placed in the ice box (0° C.), while the others were placed outdoors in glass cylinders at Hamilton, Mont.

TABLE 2.—*Studies of tick virus in unfed nymphs of lots 969c and 788—Intraperitoneal injection of tick viscera into guinea pigs, contents of one tick into each pig—All pigs surviving tick inoculation 10 days or more were given 1 c. c. of blood virus.*

Lot data.	Not incubated.		Incubated.	
	Taken from ice box Dec. 17, 1922, and inoculated immediately.		Taken from ice box Dec. 19, 1922, incubated 24 hours at 37° C., and then inoculated.	
	Tick No.	Result of inoculation.	Tick No.	Result of inoculation.
Lot 969c. Larvae infected Aug. 31, 1922. Began molting to nymphs Sept. 18, 1922. Unfed nymphs kept in ice box.	1	Spotted fever, fatal.	19	Spotted fever, fatal.
	2	Spotted fever, recovered.	20	Do.
	3	Do.	21	Do.
	4	Do.	22	Do.
	5	Immunity.	23	Do.
	6	Negative.	24	Do.
	7	Do.	25	Do.
	8	Do.	26	Negative.
	9	Do.		
	10	Do.		
	Taken from ice box Dec. 19, 1922, and inoculated immediately.		Taken from ice box Dec. 20, 1922, incubated 24 hours at 37° C., and then inoculated.	
Lot 788. Larvae infected Aug. 2, 1922. Began molting to nymphs Aug. 18, 1922. Unfed nymphs kept in ice box.	11	Immunity.	27	Spotted fever, fatal.
	12	Do.	28	Do.
	13	Do.	29	Do.
	14	Do.	30	Do.
	15	Negative.	31	Do.
	16	Do.	32	Do.
	17	Do.	33	Spotted fever, recovered.
	18	Do.	34	Do.
			35	Do.
			36	Do.
			37	Negative.

Table 2 also demonstrates the increased virulence of tick virus following incubation, in this case in unfed infected nymphs. It will be noted that some of the nonincubated nymphs of lot 969–C produced mild spotted fever, whereas when infection occurred due to inoculation with the incubated nymphs it was always fatal. Although mild infection followed injection of several of the nonincubated nymphs of the above lot, the nonincubated nymphs of lot 788 produced immunity only, closely paralleling results with nonincubated infected adults.

Charts 1, 2, and 3 have been prepared to compare the virulence of spotted-fever virus in unfed, unfed and incubated, and incubated and fed adult ticks.

Chart 1 gives the temperature curves of guinea pigs injected with one infected tick each, taken directly from the ice box. The first four tests were carried out on October 31, 1923, the last three on January 2, 1924. In five pigs no fever followed the injection. Two showed an elevation of 39.8° C. and 40° C., respectively, for one day

each.[6] The subsequent injection of blood virus was negative in two pigs, indicating complete immunity. The others developed mild fevers. The results here are similar to those obtained when unfed, hibernated adults from nature are tested by injection into guinea pigs.[7]

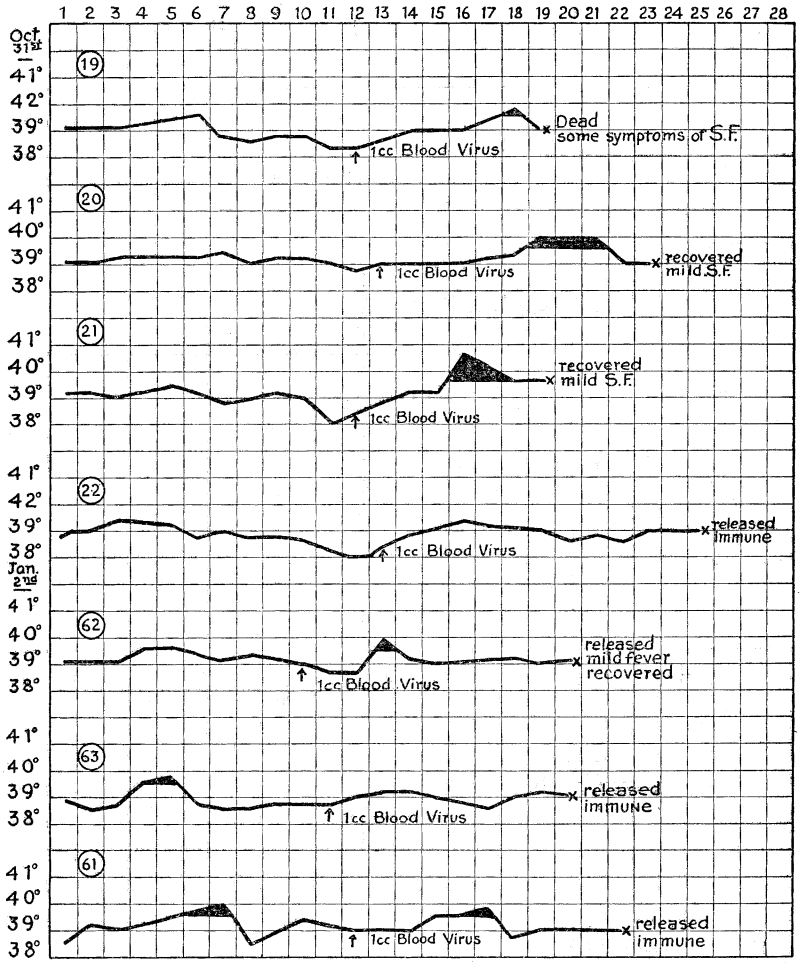

CHART 1.—Lot 2351-B-(2A). Unincubated and unfed ticks injected immediately upon removal from ice box.

The tests of Chart 2 were identical with those of Chart 1, except that in the former the ticks were incubated 24 hours at 37° C. All the guinea pigs developed spotted fever with typical external lesions and survived 10 days or more.

[6] We have considered any temperature in guinea pigs above 39.6° C. to be a definite fever, and areas in the chart lying between this line and the temperature curve are shaded in black. While some investigators consider 39.2° C. to be the upper limit of a normal guinea pig's temperature, it is believed the temperature varies considerably with that of the surrounding air and the age of the animal. Young pigs run a consistently higher temperature than those which have matured.

[7] Spencer, R. R., and Parker, R. R.; Loc. cit.

Chart 3 shows temperature curves of guinea pigs Nos. 87, 88, 90, and 94, upon each of which two ticks fed for three days (the arrows indicate the day on which the ticks were removed), and those of guinea pigs 87–A and 87–B, 88–A and 88–B, 90–A and 90–B, 94–A and 94–B, which were injected with the viscera of the ticks after

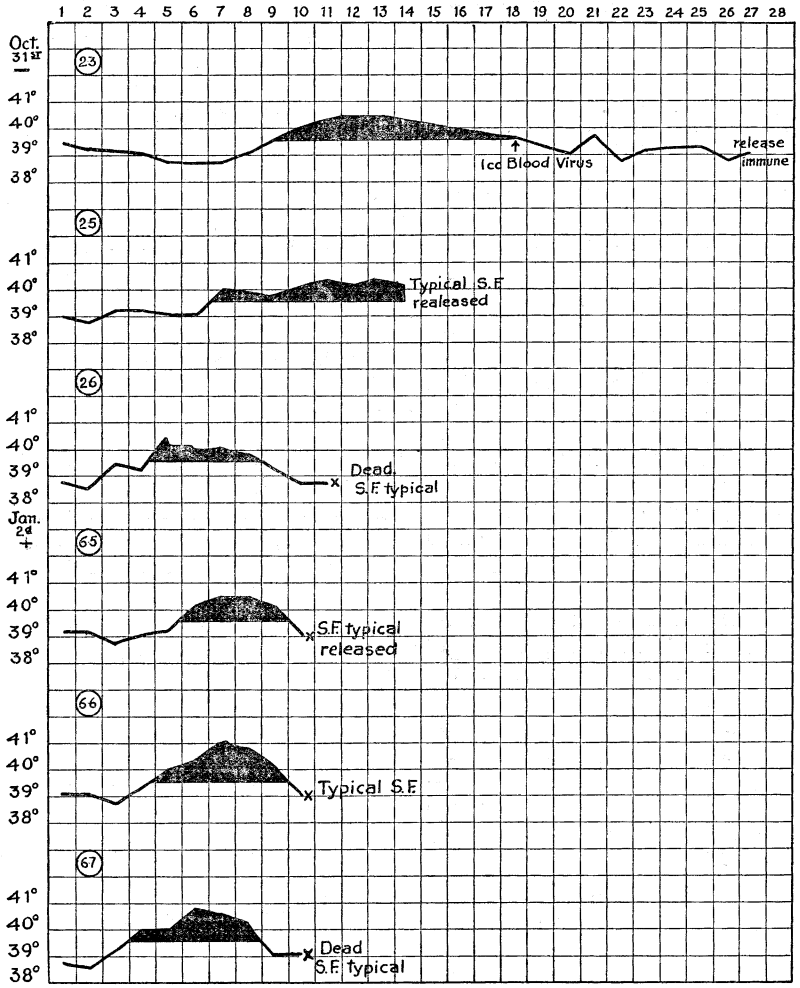

CHART 2.—Lot 2351–B–(2A). Unfed ticks removed from ice box, incubated 24 hours at 37° C., and injected peritoneally

removal. All 12 guinea pigs died, none surviving the tenth day, the majority dying within eight days, and one as early as four days. Post-mortem examination revealed the lesions of spotted fever in all cases, although five of the pigs ran practically an afebrile course, the temperature never exceeding 39.6° C. In the case of pig A–87, which died on the fourth day without elevation of temperature, two fresh pigs were inoculated intraperitoneally with an emulsion

of the spleen. Both animals ran a fever and showed scrotal lesions of spotted fever.

Chart 4 presents the temperature curves of three guinea pigs upon which there fed, respectively, 10, 13, and 9 ticks from the uninfected control lot 1988–E. After three days' feeding they were removed and their emulsified viscera injected intraperitoneally into their respective hosts. No elevation of temperature followed. After

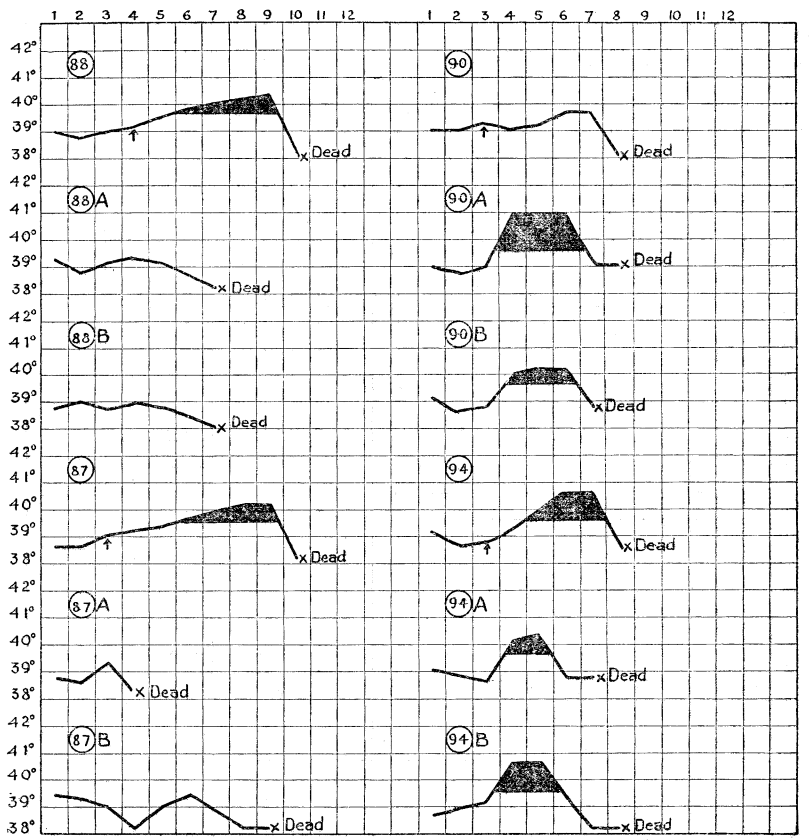

CHART 3.—Lot 2351-B-(2A). Ticks removed from ice box, incubated 24 hours at 37° C., then fed 3 days and injected intraperitoneally.

12 days 1 c. c. of blood virus was given and was followed by typical spotted fever. This chart shows that a large number of uninfected fed ticks will not kill guinea pigs either by feeding or intraperitoneal injection of their viscera after feeding, nor will such injections immunize.

Comparative studies of Charts 1, 2, and 3 reveal remarkable differences. Of 7 guinea pigs inoculated with *unfed, unincubated infected* ticks (Chart 1), not one developed typical spotted fever, although the presence of the virus was indicated by the results of

subsequent immunity tests, some guinea pigs being entirely and some partly immune. Of 6 guinea pigs injected with *incubated infected* ticks (Chart 2), all developed spotted fever, but survived 10 days or longer. Of 4 guinea pigs on which 2 *incubated infected* ticks each were allowed to feed (chart 3), all died of spotted fever in 10 days or less, and of 8 guinea pigs into which these identical *fed* ticks were inoculated, all died in 4 to 9 days. These differences are observed in a lot of ticks, the progeny of a single female. They were infected on the same host at the same time and subsequently kept in the same environment until the beginning of the test. Virulence of low grade is manifested in *unfed, unincubated* ticks taken directly from the ice box; virulence sufficient to produce definite spotted fever

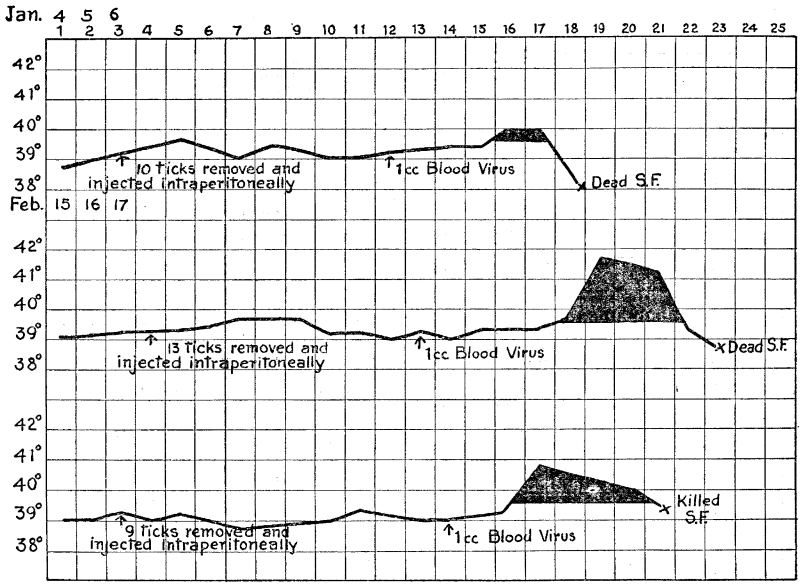

CHART 4.—Lot 1988-E. Noninfected control ticks.

but no early deaths is seen in the *unfed, incubated* ticks, and a virulence of high degree in ticks *incubated* 24 hours at 37° C. and then *fed* for three days on a guinea pig. In brief, a progressive development of the virulence has taken place. Nothing comparable to this is seen in a strain of tissue virus, the virulence of which remains fairly constant for months.

Many more tests aside from those given in charts 1, 2, and 3 were performed. Some ticks of this infected group (2351–B) failed to give infection, but no results were obtained inconsistent with those outlined in the charts. Chart 4, however, represents the total number (32) of uninfected ticks tested as controls.

Chart 5 represents the results of injecting guinea pigs on January 5, 1924, with various dilutions of a tick virus emulsion. The viscera

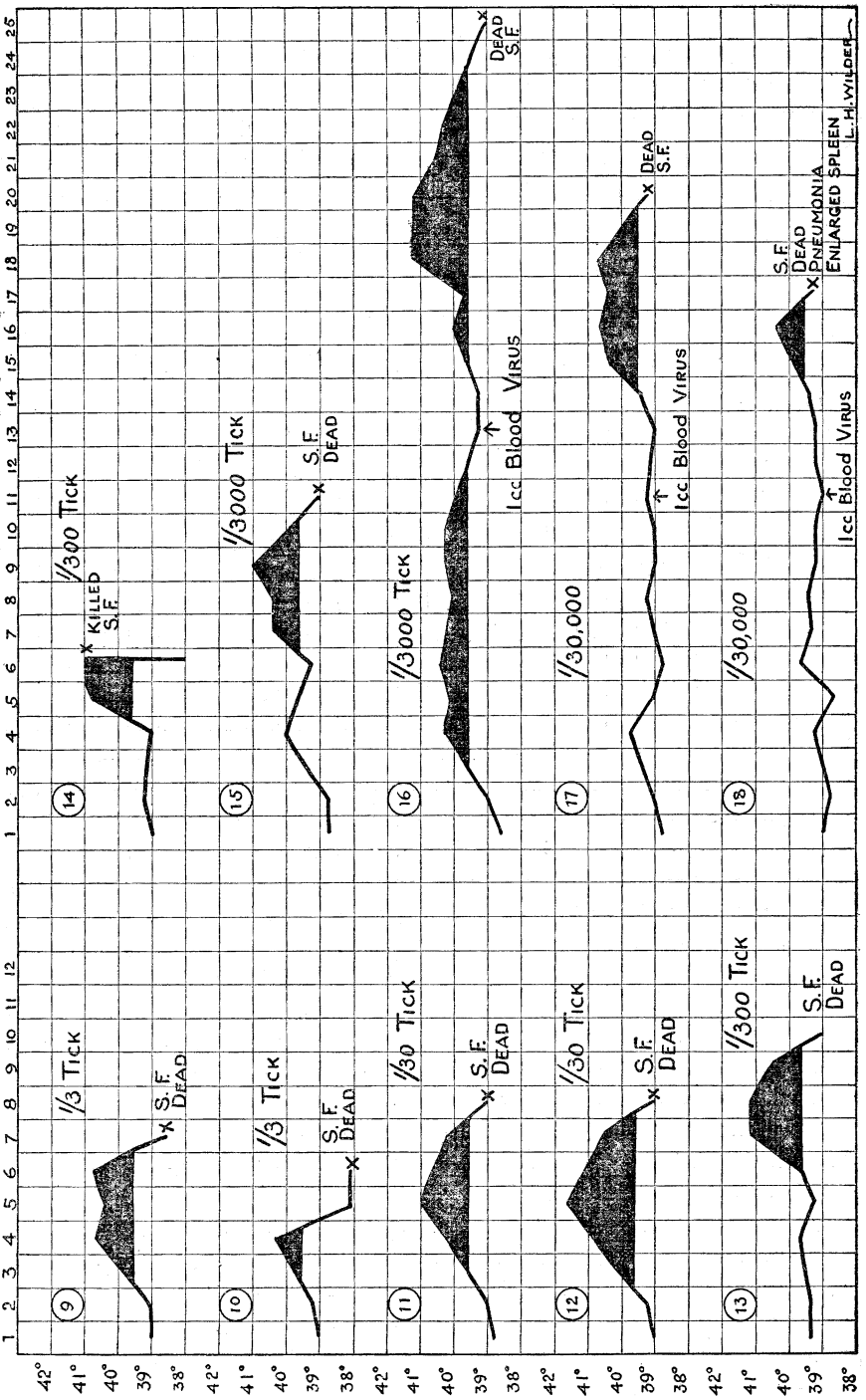

CHART 5.—Titration of virus in fed ticks.

of infected adults of lot 2351–B were ground in a mortar with a small amount of salt solution. Dilutions were made so that 1 c. c. of each represented a definite fractional part of a tick and each such fraction was inoculated into two guinea pigs. All guinea pigs receiving from 1/3 to 1/3,000 of a tick developed spotted fever, except one animal that received the latter dilution. Although this guinea pig developed fever, it was shown not to be spotted fever by the subsequent immunity test. Both 1/30,000 dilutions were negative. Subsequent titrations of virus from the same tick lot made on March 28, 1924, gave an infectious dose as low as 1/5,000 of a tick.

The contents of one adult tick after three days' feeding (the same as used for titrations) weighs about 0.01 gram and therefore 5,000 M. I. D. (minimum infectious dose) of tick virus may be concentrated in this amount of recently fed tick tissue. The tick has served, therefore, as a more efficient culture media than the guinea pig, the blood of which is infectious in minimal doses of from 1/100 to 1/1000 c. c. On this basis, tick virus of adult ticks when reactivated by freshly ingested blood may contain, volume for volume, 500 to 5,000 times as many M. I. D. as guinea pig serum virus.

Titration of tick virus from 15 lots of engorged larvæ and 11 lots of engorged nymphs indicates that they do not contain as potent a virus as that found in the recently fed adults. The difficulties experienced in rearing rabbit ticks (*Haemaphysalis leporis-palustris* Packard) have thus far prevented titration of the virus of this species, which is, perhaps, an equally important factor in the maintenance of spotted fever in nature as *Dermacentor andersoni*.

On the same date (March 28, 1924) that titrations of lot 2351–B tick virus gave 1/5000 of a *fed* adult tick as the M. I. D., control tests with *unfed* adults of the same lot were made in order to exclude a spontaneous increase in virulence and infectiousness in the unfed ticks during their long exposure to a cold environment without the stimulus of heat or blood (subsequent to the tests made on October 31 and January 2, see Chart 1). Eight *unfed* and *unincubated* ticks of this lot kept outdoors at Hamilton, Mont., all winter were injected into eight guinea pigs on March 28, 1924. None of these died or developed a typical spotted fever, but three were immune to a subsequent injection of blood virus. This result was similar to that secured by a similar test on four of these ticks on October 31 of the previous year (Chart 1), and, therefore, indicate that the results of the titration as made on January 5 (Chart 5) and March 28, 1924, were due to a reactivation of the tick virus by *incubation* and *feeding* (titration of January 5, Chart 5) and feeding alone (March 28) in the respective instances. It should be stated that on January 5 the ticks would not feed without incubation, whereas on March 28 incubation was not necessary. Feeding alone is apparently sufficient

to bring the virus to its highest virulence and concentration, and it is necessary to resort to previous incubation only during the winter months, when ticks usually refuse to feed.

2. INJECTION OF PHENOLIZED TICK VIRUS.

Chart 6 gives the temperature records of 10 guinea pigs inoculated subcutaneously with 1 c. c. of infected, fed, adult tick viscera emul-

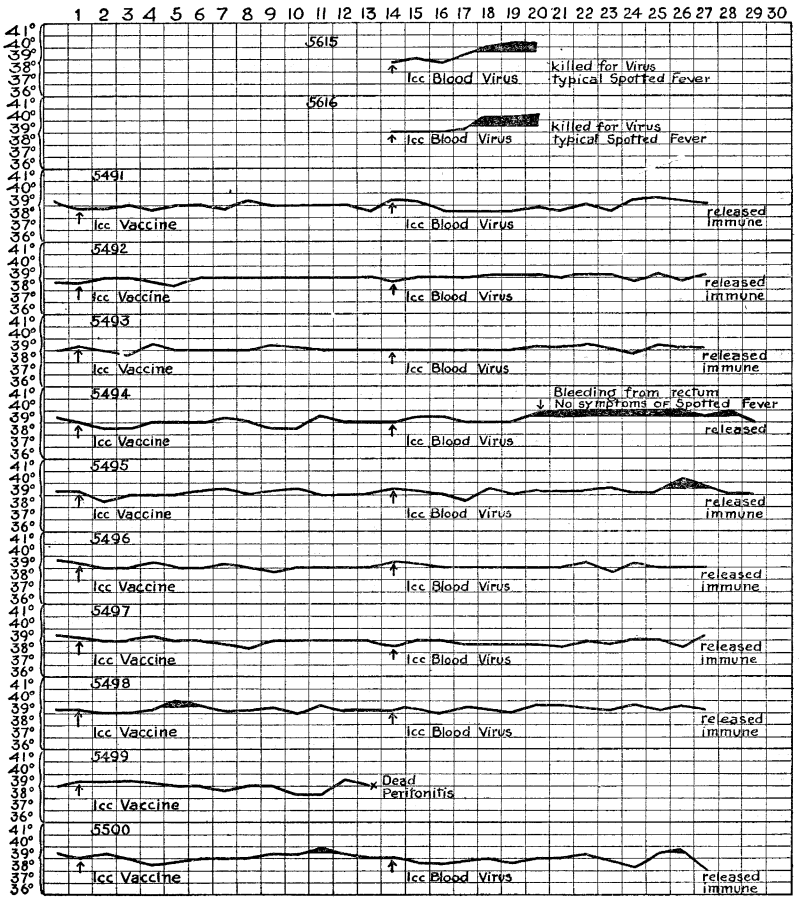

CHART 6.—Injection of phenolized tick virus.

sified in salt solution, the virus having been killed by the addition of 0.5 per cent phenol. The "vaccine" was prepared so that each cubic centimeter contained the equivalent of one tick. Before being used, it had remained in the ice box over 20 days. Titration of the virus before the addition of phenol gave a M. I. D. of 1/5000 tick. Therefore each animal received 5,000 infectious doses of killed virus. Guinea pigs Nos. 5615 and 5616 were control animals which demonstrated the infectiousness of the blood virus given on the fourteenth

day after inoculation. Guinea pig No. 5495 gave a fever of short duration on the eleventh day following the immunity test, but there was no evidence of spotted fever. Only two guinea pigs, Nos. 5498 and 5500, had elevation of temperature following vaccination. Their temperatures each reached 40° C. for one day and were probably not due to the vaccine.

The table indicates that the killed virus in the contents of one fed, infected tick is sufficient to protect guinea pigs. Vaccine preparations from other infected tick lots have never failed to protect when the same amounts are used, and therefore further concentration of the material has not thus far been attempted. The duration of this immunity, the minimal immunizing dose, and the period following vaccination before immunity is established are not yet known. The feasibility of human vaccination also naturally arises. In this connection, the relative harmlessness of tick material is suggested by the absence of secondary infection following the intraperitoneal injection into guinea pigs of the macerated viscera of 32 uninfected ticks after feeding (Chart 4) and in the one instance in which the vaccine has been administered subcutaneously to man (9,500 M. I. D. of killed virus), slight local and no constitutional reaction followed.

3. COMPARISON OF TICK AND TISSUE VIRUS.

Breinl,[8] in recent studies on the virus of typhus fever in lice, has emphasized the characteristics of louse virus as distinguished from animal-tissue virus. He observed in animals inoculated intraperitoneally with louse virus a shorter period of latency, a more irregular fever, and a higher death rate than among animals inoculated with tissue virus, and concluded that the irregular fever was due to the effect of large quantities of dead virus in the presence of live virus. Further, he concluded that the louse tissue contained far in excess of 100,000 doses of dead virus because of the inability to produce immunity with an amount of dead guinea-pig virus which contained this number of M. I. D. The observed differences between tissue virus and louse virus in typhus are seen to be somewhat analogous to those found by us between tick virus and guinea pig virus in Rocky Mountain spotted fever. However, in interpreting our results we do not believe that the atypical infection following the use of tick virus is due to the combined action of dead and living organisms. We are rather inclined to the view that a decided change of the virus in quality or quantity or both has taken place. The afebrile and fatal infection following tick feeding *as well as injection of tick contents* (Chart 3) can not be readily accounted for on the ground of an interaction between live and dead virus, for it is highly improbable that

[8] Breinl, F.: Studies of Typhus Virus in the Louse. Jour. Inf. Dis., Vol. 34, No. 1, Jan., 1924.

large quantities of dead virus can be injected into an animal by means of tick feeding. We have recently observed in rabbits also a highly fatal infection following the feeding of infected ticks upon them. Yet, as every one working with Rocky Mountain spotted fever knows, it is extremely rare to observe a fatal outcome in rabbits following the inoculation of the usual laboratory strain preserved by passage through guinea pigs.

In contrast with the mammalian host, the stages in the life cycle of the tick must influence the life of the contained virus, which strongly suggests the existence of a cycle in the life of the virus also. Phases of this cycle are herein indicated by the variations in virulence and infectivity (Charts 1, 2, and 3) of tick virus and the variation implied in the fact that killed-tick virus possesses strong immunizing power never exhibited by killed-tissue virus.

SUMMARY.

1. In confirmation of earlier observations of previous workers, ticks of the species *D. andersoni* which have received the infection of Rocky Mountain spotted fever in the larval or nymphal stage retain it in the adult stage.

2. A 24-hour incubation at 37° C. of unfed hibernating nymphs and adults infected as larvæ and subsequent injection of emulsions of such ticks into guinea pigs give a higher percentage of positive infection than the injection of similar ticks not incubated.

3. Infection of Rocky Mountain spotted fever in adult ticks subjected to winter temperatures (32° F. or below) may be demonstrated by the production of immunity in guinea pigs following the injection of tick viscera immediately upon removal from cold temperatures, by a moderate but typical spotted fever following the injection of ticks after 24 hours incubation at 37° C., and by virulent spotted fever following tick feeding or the injection of ticks after feeding.

4. Control adult ticks free from all infection do not produce death or illness in guinea pigs by feeding nor by injection of such ticks after feeding.

5. One infected adult tick may contain after feeding, from 3,000 to 5,000 M. I. D. for a guinea pig.

6. Emulsion of infected fed adult ticks treated with 0.5 per cent phenol will protect guinea pigs against 1 c. c. of blood virus.

7. Nothing in the behavior of blood or tissue virus is comparable to the changes observed in tick virus.