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# Psychiatric Institute

## A FILTER-PASSING VIRUS OBTAINED FROM DERMACENTOR ANDERSONI.

By HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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In the course of a bacteriological study of *Dermacentor andersoni*, the wood tick concerned with the transmission of Rocky Mountain spotted fever<sup>1</sup> and of tularemia,<sup>2</sup> a filter-passing virus was met with and, apparently, carried through seven generations *in vitro*, as well as through experimental animals. The history of the tick material with which these studies were made is as follows:

### *Source of the Virus.*

The tick from which the virus was isolated was one of a lot of 51 "drag" ticks collected in Saw Tooth Canyon, Montana, in the spring of 1925 and sent me through the courtesy of Dr. R. R. Parker, of the United States Public Health Service. Four ticks of this lot, after having been kept in the refrigerator (10–12°C.) for 54 days, were placed for 48 hours at 37°C. preparatory to tests for infection with spotted fever virus, according to the procedure of Spencer and Parker.<sup>3</sup> The tests were first made by allowing the ticks to feed for 72 hours, each on a normal guinea pig. Ticks 1, 3, and 4 became engorged with blood; Tick 2 was found dead. The guinea pig on which Tick 1 had fed developed typical spotted fever, the others remained well. The ticks were then eviscerated, the viscera were individually suspended in 4 cc. of salt solution, and 1 cc. of each suspension was injected into a normal guinea pig. The suspensions of Ticks 1 and 2 induced typical spotted fever, that of Tick 4 produced no reaction. The sus-

<sup>1</sup>Ricketts, H. T., *J. Infect. Dis.*, 1907, iv, 141.

<sup>2</sup>Francis, E., *J. Am. Med. Assn.*, 1925, lxxxiv, 1243.

<sup>3</sup>Spencer, R. R., and Parker, R. R., *Pub. Health Rep., U. S. P. H.*, 1924, xxxix, 3027.

pension of Tick 3, which contained an unusually large number of microorganisms very similar to those found in infected ticks, induced a fever resembling spotted fever but one which conferred no immunity to the spotted fever virus. It is this infective agent existing in Tick 3 with which the present report is concerned.

Simultaneously with the injections into guinea pigs, each tick suspension was inoculated into special culture media, which are described in another paper,<sup>4</sup> and duplicate series of tubes were kept at 26°C. and at 37°C. The presence of ordinary bacterial contamination was

TABLE I.

Tick No.	Result of feeding.	Result of inoculation.
1	Typical spotted fever.	Suspension (containing moderate number of organisms similar to those in suspension of Tick 3) induced typical spotted fever.
2 (found dead).	— (Perhaps never attached.)	Suspension (containing some microorganisms) induced typical spotted fever.
3	— No immunity developed.	Suspension (containing unusually large numbers of microorganisms) induced fever very similar to that of spotted fever but none of the skin lesions of the disease. Immunity test, 17 days after inoculation, negative.
4	— No immunity developed.	Suspension (in which no microorganisms could be demonstrated) produced no reaction. No immunity developed.

controlled by the use of plain broth and agar slants. The culture made with the suspension of the dead tick, Tick 2, was discarded because of mold contamination. Cultures made from the suspensions of Ticks 1, 3, and 4 were tested on guinea pigs at various intervals, beginning as early as 72 hours of incubation. The amount of the culture used for inoculation was 0.1 cc. or less and was usually suspended for convenience in 1 to 2 cc. of saline.

No fever or other reaction developed in guinea pigs which had

<sup>4</sup> Noguchi, H., *J. Exp. Med.*, 1926, *xliii*, 515.

received the 72 hour cultures of Ticks 1 and 4. When tested with spotted fever virus 18 days after the inoculation, the animals inoculated with the Tick 4 cultures developed spotted fever, those which had received Tick 1 cultures proved immune. No real culture, however, was obtained with the suspension of Tick 1; it was completely inert when tested again 25 days later.

The cultures made with the suspension of Tick 3, however, gave rise to exactly the same type of fever in guinea pigs as had the original material. The 72 hour cultures proved active, and as time went on their activity gradually increased. As no microorganisms could be detected by dark-field examination or by any staining method, the infective agent present in the culture will be referred to here as a virus.

The virus is aerobic; it is capable of growing at 26°C. and 37°C. and has been continued in subcultures for seven generations at 37°C. It does not grow in ordinary broth or on slant agar. It produces a slight turbidity when grown in serum media containing certain carbohydrates (mixed sugars). No putrefactive odor or gas is produced. The blood and spleen,—the latter organ always much enlarged, dark red, and soft,—of guinea pigs responding to the cultures of the virus are infective, and continuous passages from animal to animal up to the 7th generation have been maintained with the material taken on the 3rd, 4th, and 5th days of fever. No microorganisms have been seen in cultures made with the blood or spleen of such animals. The virus was recovered from the blood of infected guinea pigs. It proved to be filterable through Berkefeld N filters.

Chart 1 shows the type of temperature curve observed in infection of guinea pigs with the virus under discussion. The result of an immunity test with spotted fever blood after recovery is also indicated in the chart.

#### EXPERIMENTAL.

##### *Original Material.*

B-C Tick 3, fed for 72 hours on guinea pig infected with spotted fever virus, Barlow-Cooper strain. 54 days later, fed on normal guinea pig 72 hours. *Result of feeding:* Animal showed no sign of infection. *Result of inoculation of suspended viscera:* Fever (Chart 1) developed 72 hours after inoculation; animal remained

febrile 7 days. Blood withdrawn at the height of fever sterile. No scrotal or ear lesions appeared, and subsequent inoculation with spotted fever virus (17 days after inoculation of tick suspension) induced the typical symptoms and lesions of the disease.

### Culture Experiments.

*1st generation.*—A normal guinea pig received 0.1 cc. of culture (special semi-solid and slant media) kept 3 days at 26°C. after inoculation with 0.1 cc. of suspension of Tick 3. After 4 days the animal developed high temperature which continued for 4 days (105, 106, 104.5, 104.4°F.). No scrotal lesions appeared. Tested for immunity by injection of spotted fever virus (guinea pig blood) after 23 days. No protection.

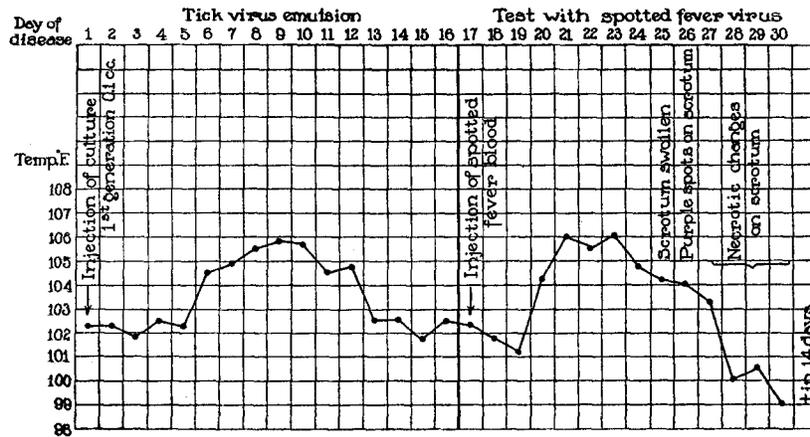


CHART 1.

Similar material, kept 3 days at 37°C., induced fever after 3 days incubation, which lasted 6 days (104.4, 105.6, 105, 103.8, 104.4, 104.2°F.). No scrotal lesions at any time. No immunity to spotted fever virus, as shown by immunity test 31 days after culture inoculation.

The same material, after 10 days at 37°C., induced a similar febrile reaction in two guinea pigs. In one, fever appeared after 4 days and lasted 6 days (104.6, 105.6, 105.6, 105.4, 105.2, 104.6°F.). No scrotal lesion developed. The animal showed no immunity against spotted fever when tested 12 days later. The second animal reacted with fever after 4 days and was killed for examination on the 5th day of fever (104.4, 105.2, 105.6, 106, 105°). The spleen was greatly enlarged and dark red, not unlike the spleen of a guinea pig infected with spotted fever; but no other lesions were found.

After 13 days at 37°C., the same material induced after 3 days incubation a

fever lasting 7 days. No scrotal lesions developed. When tested with spotted fever virus, 15 days after the inoculation of culture, this animal escaped infection. Two other guinea pigs which were inoculated with the same culture material and also reacted with fever did not prove to be immune to the spotted fever virus.

After 17 days at 37°C., the material was tested for its virulence by injecting guinea pigs with dilutions, as shown in Table II.

A second set of cultures was made with the same suspension after it had been kept 10 days at 0°C., and the culture tubes were placed at 37°C. for 8 days. A guinea pig which received 0.1 cc. of culture developed high temperature after 3 days incubation and remained continuously febrile for 8 days (104.6, 106.4, 106, 105.2, 105.6, 105, 104.8, 104.2°F.). No scrotal lesions developed at any time.

*2nd generation.*—Subcultures, made on the same media as the original cultures, and kept for 10 days at 37°C., were injected into two guinea pigs. High temperature developed in both animals after 6 days and was continuous in one for 6 days (104.4, 104.4, 104.6, 104.8, 104.4, 104°F.) and in the other for 5 days (105, 105.4,

TABLE II.

Amount injected.	Incubation period.	Duration of fever.	Immunity tests with spotted fever virus.
cc.	days	days	
1	3	7	No protection.
0.1	4	7	" "
0.1	3	8	Test not made.
0.01	4	7	No protection.
0.001	5	4	" "
		(105.4, 105.6, 104, 104.8°F.)	
0.0001	5	7	Test not made.
		(Chart 2.)	

105.2, 104.6, 104°). No scrotal lesions appeared. The first animal was tested for immunity to the virus 16 days after the culture inoculation. It proved immune. The second was tested with spotted fever virus after the same period. It reacted typically.

A second set of subcultures was kept 9 days at 37°C. and tested in normal guinea pigs. The animals had high temperature after 5 days which continued for 6 days (104, 104, 105.4, 105.8, 106, 104°F.). No scrotal lesions developed, and no protection against the spotted fever virus could be demonstrated. After 13 days at 37°C., the material gave rise after 3 days to high fever which lasted 7 days (104, 104, 105.5, 106.8, 107, 105.5, 104.5°), but no scrotal lesions appeared (Chart 3).

*3rd generation.*—Subcultures from tubes of the 2nd generation, kept 4 days at 37°C., induced in guinea pigs, after 6 days incubation, a fever lasting 5 days (104.5, 106.5, 106, 105.5, 105°F.) (Chart 4). There were no scrotal lesions. Another set of subcultures, after 8 days at 37°C., induced mild fever after 10 days incubation.

VIRUS FROM DERMACENTOR ANDERSONI

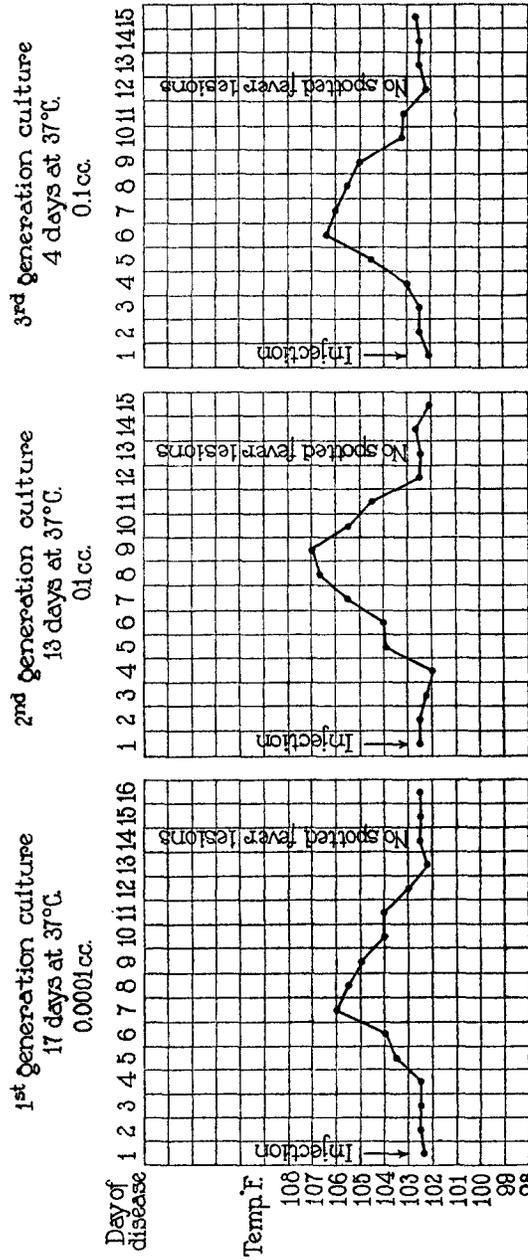


CHART 4.

CHART 3.

CHART 2.

In Charts 2, 3, and 4 are presented temperature curves of some of the guinea pigs inoculated with cultures of the 1st, 2nd, and 3rd generations.

*4th generation* cultures, 7 days old at 37°C., induced in one guinea pig after 10 days incubation a fever lasting 5 days, and in another fever of 3 days duration.

*5th generation*.—7 day old cultures, kept at 37°C., induced a febrile reaction lasting 4 days, the incubation period being 10 days.

*6th generation* cultures, 10 days old at 37°C., induced fever of 5 days duration after an incubation period of 7 days.

*7th generation*.—Subcultures from the 6th generation, after 4 days at 37°C., induced fever of 5 days duration following an incubation period of 10 days.

The number of positive reactions became less constant with further subcultures, the virus becoming gradually attenuated.

*Macacus rhesus 1*.—The monkey received an intraperitoneal injection of 1 cc. of mixed cultures of the virus, including one of the 1st generation (28 days old) and one of the 4th (8 days old). A fever fluctuating between 104°F. and 104.8° developed after 3 days and lasted 6 days. No spotted fever lesions appeared in the animal.

#### *Guinea Pig Passages.*

*1st passage*.—Guinea Pig XVIII-84B developed fever (104–105.5°F.) 5 days after receiving a 1st generation culture (13 days old at 37°C.), the temperatures recorded on successive days being 105.2, 105.4, 106, 105.5°F. The animal was killed on the 5th day of fever. The spleen was enlarged, and its appearance suggested that of the spleen in spotted fever infection. The blood, which was free from contaminating microorganisms, as tested by cultures, was injected intraperitoneally into two normal guinea pigs.

*2nd passage*.—Guinea Pig XVIII-121A<sup>5</sup> developed fever after 4 days which continued for 7 days (106.2, 105.4, 104.6, 104°F.). Guinea Pig XVIII-121B also showed high fever after 4 days (105.6, 106.4, 105.4, 105.4°F.). It was killed on the 4th day of fever for passage, cultural experiments, and virulence tests on monkeys, rabbits, and guinea pigs.

*3rd passage*.—Citrate blood from the animal just mentioned was injected into four guinea pigs, the first receiving 1 cc., the second 0.1 cc., the third 0.01 cc., and the fourth 1 cc. The fourth guinea pig received in addition 2 cc. of serum from rabbits immunized to spotted fever, of a titre such that 0.1 cc. neutralized 100 M.L.D. of spotted fever virus (guinea pig blood). The incubation period in each instance was 7 days, and the duration of fever was 2 to 3 days in the first three animals and 6 days in the fourth. There was no evidence of any neutralizing effect on the virus by spotted fever antiserum.

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<sup>5</sup> Twenty-two ticks were placed on this animal on the 3rd day of fever and allowed to feed 5 days; nineteen became engorged, three were found dead.

The same blood was injected into *Macacus rhesus* 2 and a rabbit intraperitoneally, each receiving 2 cc., but no reaction followed in either instance.

After the 3rd passage, successive transfers were made as routine, two guinea pigs being used in each passage, with two sets of cultures set up for each, until the 8th passage, 3 months after the 1st, when the work was discontinued. No increase in virulence was noted, but on the contrary the duration of fever tended to become shorter and the incubation period longer. At each passage the blood was tested for ordinary bacterial invasion, as for example by bacilli of the paratyphoid group; no such invasion was discovered.

#### *Filtration Experiments.*

Cultures of the 1st, 2nd, and 3rd generations, grown on the special semisolid and slant media, were pooled and diluted with an equal volume of 0.9 per cent salt solution. The suspension, which contained no demonstrable microorganisms, was thoroughly shaken and passed through a Berkefeld filter N. The clear, light brownish filtrate was injected into four guinea pigs in amounts of 4, 3, 2, and 1 cc., respectively. The first three animals developed, after 4 days incubation, typical fever (104.5–105.5°F.) which was continuous for 7 days; the guinea pig which was inoculated with 1 cc. showed slight fever for 2 days. These animals were tested for immunity by injections of virulent spotted fever blood, but all had typical severe infections.

A guinea pig which had been injected as control with 1 cc. of the unfiltered suspension became ill in 4 days and passed through a course of fever lasting 5 days (106.2, 105.6, 106, 104.6, 104.6°F.).

Subcultures made with the filtrate yielded an active culture, which was subsequently tested for filterability, with positive results. Neither filtrate nor cultures contained any demonstrable microorganisms.

The question arose whether the infective agent revealed by the inoculation of guinea pigs had not merely survived in the culture tubes at 37°C. The original suspension, preserved at 3°C., was tested in guinea pigs in various dilutions on the 7th and 17th days after its preparation. The results, which are recorded in Table III, show that 1 cc. of the original material induced infection, but not 0.1 cc. or less. The culture of the 1st generation, on the other hand, when 17 days old was effective in a dose of 0.0001 cc.

As has already been mentioned, the original suspension contained an enormous number of minute, non-motile, Gram-negative microorganisms resembling spotted fever organisms. These did not grow on the media employed for cultivating the filterable virus under discussion.

*Transmission of the Virus from Infected Guinea Pigs to Ticks and Vice Versa.*

*Experiment 1.*—Seven ticks which had been fed for 5 days on a guinea pig in the febrile stage of the virus infection were placed separately on seven normal guinea pigs and allowed to feed for 5 days. In one of the animals fever developed after 8 days (104–105°F.) and lasted 4 days. The others escaped infection.

The tick which had induced infection was killed 119 days later and a portion of the emulsified viscera inoculated into a guinea pig. Fever (104.5–105.5°F.) developed after 48 hours and lasted 8 days. Death occurred on the 11th day. There were no lesions of spotted fever. The spleen was soft and considerably enlarged. No bacteria could be demonstrated in cultures made from the blood and spleen. Inoculation of blood withdrawn during the febrile stage induced the usual prolonged febrile, non-fatal reaction in guinea pigs; no spotted fever lesions developed.

TABLE III.

Amount of tick suspension.	Kept 7 days at 3°C.	Kept 17 days at 3°C.
cc.		
1	Usual fever.	Usual fever.
0.1	No reaction.	No reaction.
0.01	“ “	“ “
0.001	“ “	“ “

Smears of the tick revealed only a few microorganisms, but sections showed a large number of minute Gram-negative elements in the lumen of the intestine as well as in the viscera. Cultures made with the suspension yielded no growth.

*Experiment 2.*—A female tick, which had failed to induce infection by biting, survived 110 days and laid several lots of eggs. The tick and 40 of the eggs were separately suspended in saline, and a portion of each was injected into guinea pigs. No fever developed. In the smears and sections of the tick and eggs were found a number of Gram-negative microorganisms, and cultures yielded a growth of *B. rickettsiformis*.<sup>4</sup> The finding was of interest in showing hereditary transmission of this non-pathogenic microorganism, which had already been found in the ovaries and egg cells of ticks.

*Experiment 3.*—Four ticks of a lot of eleven which had been fed for 5 days on a guinea pig infected experimentally were still living after 124 days and were separately fed on guinea pigs, but no fever was induced. The remaining seven ticks were dead but only partly dried, and a single suspension in salt solution was made of their viscera. The guinea pig injected with a portion of this suspension developed after 8 days incubation a fever of 5 days duration. The blood withdrawn on the 4th day of fever contained no contaminating bacteria. The filtrate of the emulsion failed to induce infection.

This survival of the virus in ticks for several months at refrigerator temperature is not surprising, since it occurred also in culture. A guinea pig receiving 0.2 cc. of 1st generation culture of the virus, preserved at 2°C. for 5 months, developed the typical febrile reaction after 4 days and remained febrile for 6 days, when it was killed for transfer and culture. There was no bacterial invasion.

The foregoing experiments show that the virus is taken up by ticks from the blood of the infected guinea pig, but that transmission of the infective agent to other guinea pigs by the bite of infected ticks seldom occurs under the conditions described. The presence of the virus in ticks can be demonstrated more definitely by inoculation of guinea pigs with tick emulsions. That all ticks which are fed on infected guinea pigs do not take up the virus, however, is shown by the negative results in some instances.

#### *Cross-Neutralization Experiments.*

It has been repeatedly noted that guinea pigs which have responded to the virus under discussion do not acquire immunity against the spotted fever virus. Conversely, guinea pigs which have passed through the spotted fever infection were found to be quite as susceptible to this virus as normal animals. A potent spotted fever immune serum failed to neutralize the cultured virus.

#### SUMMARY.

An invisible, filter-passing virus, pathogenic for the guinea pig, and capable of cultivation on special media for at least seven generations, has been isolated from a tick of the species *Dermacentor andersoni*. One of two monkeys (*Macacus rhesus*) inoculated became infected, and in one rabbit the result was negative.

The virus has been transmitted from infected guinea pigs to ticks as also in one instance by tick feeding from an infected tick to a guinea pig. The presence of the virus in the tick is more easily demonstrated by the inoculation of guinea pigs with a suspension of the tick viscera. Continuous high fever (104.5–106.5°F.) and enlargement of the spleen are the chief symptoms of the infection in guinea pigs. After the febrile attack the guinea pigs are not susceptible to reinoculation with the cultured virus but are subject to infection with the virus of spotted fever, and *vice versa*.