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IMMUNITY STUDIES OF ROCKY MOUNTAIN SPOTTED FEVER.

I. USEFULNESS OF IMMUNE SERUM IN SUPPRESSING AN IMPENDING INFECTION.

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Rocky Mountain spotted fever has been for the past 20 years the subject of study by various workers, including members of the United States Public Health Service. The discoveries of Wilson and Chowning,¹ McCalla,² King,³ Ricketts,⁴ Maver,⁵ Moore,⁶ Cooley,⁷

¹ Wilson, L. B., and Chowning, W. M., The so-called "spotted fever" of the Rocky Mountains, *J. Am. Med. Assn.*, 1902, xxxix, 131; Studies in *Piroplasmosis hominis*. "Spotted fever" or "tick fever" of the Rocky Mountains, *J. Infect. Dis.*, 1904, i, 31.

² McCalla, L. P., Direct transmission from man to man of the Rocky Mountain spotted (tick) fever, *Med. Sentinel*, 1908, xvi, 87.

³ King, W. W., Experimental transmission of Rocky Mountain spotted fever by means of the tick. Preliminary note, *Pub. Health Rep.*, U. S. P. H., 1906, xxi, 863.

⁴ Ricketts, H. T., The transmission of Rocky Mountain spotted fever by the bite of the wood-tick (*Dermacentor occidentalis*), *J. Am. Med. Assn.*, 1906, xlvi, 358; Further observations on Rocky Mountain fever and *Dermacentor occidentalis*, 1067; The rôle of the wood-tick (*Dermacentor occidentalis*) in Rocky Mountain spotted fever, and the susceptibility of local animals to this disease—a preliminary report, 1907, xlvi, 24; Further experiments with the wood-tick in relation to Rocky Mountain spotted fever, 1278; Observations on the virus and means of transmission of Rocky Mountain spotted fever, *J. Infect. Dis.*, 1907, iv, 141.

⁵ Maver, M. B., Transmission of spotted fever by other than Montana and Idaho ticks, *J. Infect. Dis.*, 1911, viii, 322; Transmission of spotted fever by the tick in nature, 327.

⁶ Moore, J. J., Time relationships of the wood-tick in the transmission of Rocky Mountain spotted fever, *J. Infect. Dis.*, 1911, viii, 339.

⁷ Cooley, R. A., Preliminary report on the wood tick. *Dermacentor sp.* Sixth Annual Report of the State Entomologist of Montana, *Montana Agric. Coll. Exp. Station, Bull.* 75, 1909, 95; Tick control in relation to the Rocky Mountain spotted fever, *Montana Agric. Coll. Exp. Station, Bull.* 85, 1911.

Parker,⁸ and Wolbach,⁹ have proved that the disease is transmitted to man by a certain wood tick, *Dermacentor venustus*, which receives the virus from infected wild rodents, principally gophers or Columbian ground squirrels and jack-rabbits (Wilson and Chowning; Parker). Ricketts¹⁰ was the first to transmit the disease from man to guinea pigs and monkeys by injecting the blood drawn from spotted fever patients and, after having fed normal ticks on the infected guinea pigs, to reproduce the disease again in normal guinea pigs by allowing the infected ticks to feed on the animals. The virus of Rocky Mountain spotted fever can be maintained in the laboratory for an indefinite time by animal passage, which makes its experimental study possible. Several strains of the passage virus¹¹ maintained in the guinea pig were employed in the present series of studies on immunity.

Ricketts and Gomez¹² found that the serum from guinea pigs which had recovered from the disease would protect normal guinea pigs from infection when administered within 2 to 3 days after the injection of the infecting material. The strength of the serum was such that 0.3 to 0.5 cc. was required to protect against 1 cc. of virus (1,000 infecting doses), and these investigators were unable to enhance the

⁸ Parker, R. R., Tick control program for 1919, *Montana State Bd. Entomol., Circular 1*, 1919; The control of Rocky Mountain spotted fever in the Bitter Root Valley, *Montana State Bd. Entomol., Circular 1 (Revised)*, 1921. 3rd Biennial Rep., *Montana State Bd. Entomol., 1917-18*, 1919; 4th Biennial Rep., *Montana State Bd. Entomol., 1919-20*, 1921.

⁹ Wolbach, S. B., Studies on Rocky Mountain spotted fever, *J. Med. Research*, 1919-20, xli, 1.

¹⁰ Ricketts, H. T., The study of "Rocky Mountain spotted fever" (tick fever?) by means of animal inoculations. A preliminary communication, *J. Am. Med. Assn.*, 1906, xlvi, 33.

¹¹ I am indebted to Dr. L. D. Fricks, of the United States Public Health Service, Dr. S. B. Wolbach, of Harvard Medical School, and Dr. R. R. Parker, of the Montana State Board of Health and United States Public Health Service, for their cooperation in supplying me with virus and ticks on numerous occasions since 1916.

¹² Ricketts, H. T., and Gomez, L., Studies on immunity in Rocky Mountain spotted fever. The preventive and curative action of a serum for spotted fever, and the inefficiency of sodium cacodylate as a curative agent for this disease in guinea-pigs, *J. Infect. Dis.*, 1908, v, 221.

potency by repeatedly injecting an immune animal with virus. They accordingly drew the tentative conclusion that the usefulness of the serum in human cases would be practically nil, especially since the amount required would be prohibitive when translated into terms of human body weight. Ricketts' work was not completed, however, and there seem to be no experimental data to indicate how a serum of this kind might be developed so as to be of practical value. The purpose of the experiments recorded here was to study this problem and if possible to produce an immune serum of at least sufficient potency to be useful when the time of infection is definitely known and the injection of serum can be made soon after the inoculation of the virus, as when a laboratory worker is bitten by an infected tick or accidentally inoculates himself with an instrument charged with infected material.

Although the serum of guinea pigs which have passed through a severe infection has a degree of neutralizing power when injected at the same time as the virus, it is not strong enough to check the course of the infection when introduced 24 to 48 hours later. In the present series of experiments, however, it has been found that the neutralizing power of the serum is considerably increased by one or two more subsequent inoculations of the virus, in citrated blood, into the convalescent guinea pig, and sera prepared in this way are capable of modifying the course of the disease into a non-fatal one or, in some instances, of suppressing the infection completely, provided the serum is administered during the incubation period.

The small size of the guinea pig precludes the possibility of utilizing its immune serum for human cases, but the principle developed from the experiments with the serum of guinea pigs suggested that larger animals might be capable of furnishing an equally potent immune serum in correspondingly greater amounts.

Ricketts and Gomez,¹² and later Heinemann and Moore,¹³ reported having infected horses by injecting large quantities of virus from guinea pigs or monkeys. Although the horses showed some fever on the 3rd or 4th day after inoculation, it never reached 104°F. and usually lasted only 1 day. The serum from the horses, however, was stated to possess some neutralizing power as compared with normal horse serum.

¹³ Heinemann, P. G., and Moore, J. J., Experimental therapy of Rocky Mountain spotted fever, *J. Infect. Dis.*, 1912, x, 294.

EXPERIMENTAL.

The writer's experiments with horses have so far been unsuccessful. Two adult horses were given intravenous injections of blood from infected guinea pigs in doses ranging from 20 to 50 cc. at intervals of 3 to 5 days, and a colt 6 months old received similar injections of larger amounts (35 to 95 cc.) of the virus. The rise of temperature to 103–104°F. which occurred on the 3rd or 4th day after inoculation was interpreted as being due to the introduction of a large quantity of blood corpuscles from a foreign species. The animals never showed symptoms suggestive of spotted fever, and the serum on test was found to have only slight neutralizing power for the virus; 1 cc. was necessary to neutralize about 10 M.L.D. when administered simultaneously.

In view of the facts that adult ticks feed on horses and cattle, and that a certain number of ticks collected at random prove to be infective, it was thought of interest to test the serum of horses which had been pastured in infected districts. Serum from a horse which had been pastured for several years in the Bitter Root Valley was obtained¹⁴ and tested against the virus. As shown in Table I this serum (No. 1) in a dose of 3 cc. gave complete protection against 1 cc. (approximately 100 M.L.D.) of the spotted fever virus when injected simultaneously. Of the other three sera subsequently obtained from horses pastured in the same region, one (No. 2), which was contaminated on arrival, failed to give any protection in a dose of 3 cc., while Sera 3 and 4 had definite neutralizing power, the animals receiving 3 cc. of the serum simultaneously with 100 M.L.D. of the virus recovering after a typical febrile reaction without scrotal or skin lesions.

TABLE I.
Administration of Serum from Horses Pastured for Several Years in the Bitter Root Valley of Montana to Guinea Pigs Inoculated with 100 M. L. D. of the Rocky Mountain Spotted Fever Virus.

Serum No.	Date.	Amount.	Result.	Remarks.
	1922	cc.		
1	Feb. 3	3	No infection.	Tested for immunity after 40 days and found to be completely immune.
1	Apr. 2	3	" "	Died of intercurrent infection before test for immunity could be carried out.
2 (contaminated on arrival).	" 25	3	Typical infection.	Died in 13 days.
3	" 25	3	" fever for 4 days.	Recovered without lesions.
4	" 25	3	Typical fever for 5 days.	" " "

¹⁴ The serum was obtained through the kindness of Dr. W. J. Butler and Dr. Hadleigh Marsh, of the Montana Livestock Sanitary Board.

The only animal which yields a useful serum, both in potency and quantity, is the rabbit. In the first place, the rabbit is susceptible to the virus of spotted fever. The infection is usually non-fatal, the febrile period lasting for 6 to 9 days, though it is sometimes shorter or longer. The scrotal skin may show the typical capillary thrombosis and subsequent necrosis. After recovery the animal becomes completely resistant to subsequent infection, and 0.5 to 0.1 cc. of the serum, collected 2 weeks after disappearance of symptoms, is capable of rendering non-fatal an infection produced by 100 M.L.D. of the virus, the test animal in every instance being the guinea pig. By another intravenous injection of virulent blood, *e.g.* of 4 cc., or 400 M.L.D., the serum can be raised in potency five- to tenfold, and in some instances to such a titer that 0.1 cc. will neutralize 1,000 M.L.D. The reinforcing injection is given 2 weeks after complete recovery of the animal, and the immune serum is drawn 9 days later. A serum, whether convalescent or reinforced, is sufficiently strong for practical use when it is capable in a dose of 0.1 cc. of neutralizing 100 M.L.D. of the virus. As will be seen from the results recorded in Tables II to IV, the immune rabbit serum prevents the development of the infection or renders it non-fatal when administered within the period of incubation—the earlier the administration of the serum the more complete the suppression of the infection.

Male guinea pigs of 500 gm. body weight were used. The virus¹⁵ consisted of the citrated blood from the heart of guinea pigs on the 4th or 5th day of fever and was inoculated subcutaneously. 1 cc., equivalent to 100 M.L.D., was given in Experiment 1, 0.1 cc. (10 M.L.D.) in Experiment 2, and 0.01 cc. (1 M.L.D.) in Experiment 3. Eighteen guinea pigs were used in each series, two animals being inoculated with the immune serum immediately after the injection of the virus, and two at the end of 24, 48, 72, and 96 hours, and 5, 6, and 7 days. The remaining two animals served as controls without serum. The immune serum used was a mixture of sera from several immunized rabbits and was given in a dose of 1 cc. in Experiments 1 and 2 and 0.1 cc. in Experiment 3. The titer of the pooled serum was such that when given simultaneously 1 cc. completely neutralized 100 M.L.D.,

¹⁵ These experiments were carried out with a strain furnished by Dr. Parker.

TABLE II.

Experiment 1. Administration of Immune Rabbit Serum to Guinea Pigs Inoculated with 100 M. L. D. of the Rocky Mountain Spotted Fever Virus.
Amount of serum, 1 cc. uniformly.

Guinea pig No.	Interval before serum administration.	Symptoms before serum administration.	Symptoms after serum administration.
1A	Immediately after inoculation.	None.	None.
1B	Immediately after inoculation. <i>hrs.</i>	"	"
2A	24	"	"
2B	24	"	"
3A	48	"	Temperature rose to 104.5° F. in 7 days; remained high for 24 hrs. Recovered.
3B	48	"	High fever developed in 9 days; lasted for 4 days. No scrotal lesions. Recovered.
4A	72	"	High fever on 6th day; remained for 4 days. No scrotal lesions. Recovered.
4B	72	"	High fever for 3 days after 5th day. No scrotal lesions. Recovered.
5A	96	"	High fever continued for 5 days. No scrotal lesions. Recovered.
5B	96 <i>days</i>	Temperature 105°F. in morning. Fever for 2 days.	High fever in 7 days, lasting 48 hrs. No scrotal lesions. Recovered. No abatement of temperature. Typical course. Died in 16 days.
6A	5	High fever for a day.	Fever continued at 105-106.5°F. for 8 days. Died in 17 days.
6B	5	" " (105-107° F.) for 2 days.	No fall in temperature. Typical course. Died in 13 days.
7A	6	High fever for 2 days.	No reduction in fever. Typical course. Died in 13 days.
7B	6		

TABLE II—*Concluded.*

Guinea pig No.	Interval before serum administration. <i>days</i>	Symptoms before serum administration.	Symptoms after serum administration.
8A	7	High fever for 3 days.	Apparently no effect on the course of infection. Died in 13 days.
8B	7	" " " 3 "	No beneficial effect. Typical course. Died in 12 days.
Controls without serum.			
9A		Temperature 105°F. after 96 hrs. and remained high for 5 days; fell to subnormal on the 11th day. Scrotum, ears, and feet showed typical lesions. Died in 12 days.	
9B		Rise of temperature to 104.5°F. in 96 hrs.; continued high for 7 days. Died in 13 days.	

0.1 and 0.01 cc. modified the infection to a non-fatal one, while 0.001 cc. exerted no influence upon the course of the infection. The serum was given subcutaneously.

As Table II shows, 1 cc. of the serum, given within 24 hours from the time of introduction of a massive amount (100 M.L.D.) of the virus into the body of the guinea pig, is capable of suppressing the infection; when given later, but before 96 hours (near the time of onset of the disease), this quantity does not suppress the infection but renders it non-fatal. After 5 days, that is when the disease is already manifest, no benefit seemed to be derived from administration of the serum, the animals dying within 12 to 17 days (the controls died within 12 to 13 days).

The results in the second series (Table III) are similar, except that, owing probably to the smaller quantity of the virus used (10 M.L.D.), the infection was completely suppressed in all animals which received the serum within the first 48 hours. All those which received it after 48 hours, but within 96 hours, survived, after passing through a mild infection without any scrotal or other cutaneous lesions. Administration of the serum later than 5 days invariably failed to prevent a fatal outcome, the animals dying just as the controls did.

In the third series (Table IV), in which only 1 M.L.D. was used to infect the animals, the protective effect of the immune serum was

TABLE III.

Experiment 2. Administration of Immune Rabbit Serum to Guinea Pigs Inoculated with 10 M.L.D. of the Rocky Mountain Spotted Fever Virus.

Amount of serum, 1 cc. uniformly.

Guinea pig No.	Interval before serum administration.	Symptoms before serum administration.	Symptoms after serum administration.
10A	Immediately after inoculation.	None.	None.
10B	Immediately after inoculation.	"	"
	hrs.		
11A	24	"	"
11B	24	"	"
12A	48	"	"
12B	48	"	"
13A	72	"	High fever after 5 days; lasted only 3 days. Recovered.
13B	72	"	High fever after 5 days; lasted 4 days. Recovered.
14A	96	"	High fever developed in 7 days; lasted 2 days. No scrotal lesions. Recovered.
14B	96	"	High fever in 7 days; lasted 5 days. No scrotal lesions. Recovered.
	days		
15A	5	First sign of fever (105° F.).	Fever continued for 7 days. Typical infection. Died in 12 days.
15B	5	Beginning of fever.	High fever continued for 8 days. Died in 13 days.
16A	6	" " high fever.	Fever of typical course. Died in 13 days.
16B	6	High fever for 24 hrs.	Fever remained high for 7 days. Died in 14 days.
17A	7	" " " 48 "	No influence on the course of the infection. Died in 13 days.
17B	7	" " " 48 "	Fever for 5 days. Typical course. Died in 12 days.
Controls without serum.			
18A	Temperature 105.2°F. in 5 days; remained high for 7 days. Died in 13 days.		
18B	Temperature 105°F. in 5 days; remained high for 6 days. Died in 12 days.		

TABLE IV.

Experiment 3. Administration of Immune Rabbit Serum to Guinea Pigs Inoculated with 1 M.L.D. of the Rocky Mountain Spotted Fever Virus.
Amount of serum, 0.1 cc. uniformly.

Guinea pig No.	Interval before serum administration.	Symptoms before serum administration.	Symptoms after serum administration.
19A	Immediately after inoculation.	None.	None.
19B	Immediately after inoculation.	"	"
20A	24 hrs.	"	"
20B	24	"	"
21A	48	"	"
21B	48	"	"
22A	72	"	"
22B	72	"	"
23A	96	"	"
23B	96	"	Fever in 7 days; lasted 5 days. Recovered.
24A	days 5	"	Fever in 7 days; lasted 48 hrs. Recovered.
24B	5	Temperature 105° F. in the morning.	Fever lasted for 8 days. Typical scrotal lesions. Died in 16 days.
25A	6	Temperature 106° F. in the morning.	Fever remained high for 7 days. Typical infection. Died in 14 days.
25B	6	None.	Temperature 106°F. in 9 days; lasted 48 hrs. Recovered.
26A	7	"	Fever in 9 days; remained high 3 days. Recovered.
26B	7	Temperature 105.5° F. for 24 hrs.	Fever and scrotal lesions typical. Died in 17 days.
Controls without serum.			
27A		Temperature rose to 105.5°F. in 5 days; lasted for 9 days. Scrotal skin necrotic; ears hardened. Died in 18 days.	
27B		Temperature 105°F. in 7 days; lasted for 8 days. Scrotal lesions typical. Died in 16 days.	

much more pronounced than in the first two series. Here the infection was completely suppressed when 0.1 cc. of the serum was given within 72 to 96 hours. In one of each set of two animals treated

with the serum after 5, 6, and 7 days respectively, the infection was mild, undoubtedly because the serum was given before the animals showed any fever, while they were still in the incubation period, which is decidedly longer in the case of 1 M.L.D. than when 10 or 100 M.L.D. are inoculated.

DISCUSSION AND SUMMARY.

From the results of the experiments presented it is evident that in guinea pigs an early administration of immune rabbit serum will suppress the infection; that is, if it is given within the period of incubation, the effect being proportionately greater the earlier the serum is administered. Almost no beneficial effect is observed when the serum is given after the onset of the disease. In the animals inoculated with 10 to 100 M.L.D. the incubation period is shorter than when 1 M.L.D. is injected; nevertheless 1 cc. of the immune serum saved the animals as late as 96 hours from the time of the introduction of the virus into the system. When administered within 24 hours in the case of 100 M.L.D. and within 48 hours in the case of 10 M.L.D., the serum completely neutralized the virus, and the animals escaped infection altogether. On the other hand, the same quantity of the serum only modified the infection into a non-fatal one when given a day or two later. In the animals which were inoculated with 1 M.L.D. the incubation period was a day or two longer, and the neutralizing effect of the serum was much more powerful. Here animals were saved as late as 5, 6, and 7 days and with a much smaller quantity of the serum (0.1 cc.).

As to the usefulness of such an immune serum in human cases, the relative susceptibility of man and the guinea pig must first be considered. In a large number of experimental infections carried out with guinea pigs in the past 6 years almost never has a naturally refractory animal been encountered. The mortality is nearly 80 per cent with most strains, although as low as 50 per cent with some. The strain used in the present study caused death in nearly 80 per cent of the animals. Hence the susceptibility of guinea pigs is at least as great as that of man, in whom the mortality in the Bitter Root Valley is estimated to be about 70 per cent.^{1,9}

The relative length of the incubation period in guinea pig and in man is another point which requires analysis. In guinea pigs it varies somewhat according to the number of passages, being as short as 3 days when 100 M.L.D. or more of an adapted virus are inoculated. On the other hand, when the infection is the result of 1 M.L.D. or the bite of an infected tick, the incubation period is much longer, being 5, 6, or 7 days in the former and 7 to 8½ days in the latter instance, as with the present strain. In man the infection is brought on by the bite of an infected tick, and the period of incubation varies from 3 to 10 days but is usually 7 days; *i.e.*, it is about the same as in guinea pigs infected with 1 M.L.D. Hence we may regard the susceptibility of man and the guinea pig as nearly equal.

The final point to be considered is the quantity of the immune serum that may be recommended for use in human cases. To prevent the infection in a guinea pig weighing 500 gm., 0.1 cc. of the serum was sufficient. This quantity protected the animal against 1 M.L.D. even as late as 5, 6, or 7 days. Calculated on this basis, 16 cc. of the serum would be required for a man weighing 80 kilos (about 160 pounds); that is, 16 cc. of an immune rabbit serum, administered before onset of the disease, should theoretically be sufficient to save a man of average weight against an infection brought about by the bite of an infected tick or by a laboratory accident. It would probably be best to administer the serum intravenously. The titer of the immune serum should be previously determined in guinea pigs, and 1 cc. should neutralize 100 M.L.D. completely and 0.1 and 0.01 cc. render the infection non-fatal. Such a serum is easily produced in rabbits (a rabbit weighing 2,500 gm. will yield 50 to 60 cc. of the serum) and probably will remain active a year or longer when kept at refrigerator temperature.

CONCLUSIONS.

A potent immune serum against the virus of Rocky Mountain spotted fever can be produced in the rabbit.

By early administration of such an immune serum the virus introduced into the guinea pig can be prevented from multiplying and causing fatal infection. When given within the period of incubation the suppression of the infection is assured, but after the onset of the disease the serum has no beneficial effect.

In view of the comparative susceptibility of man and the guinea pig it is recommended that in every instance when the bite of a tick gives reason to suspect a possible infection with spotted fever, or when a person accidentally inoculates himself with the virus, about 0.2 cc. of the serum per kilo of body weight (or 0.1 cc. per pound) be injected immediately, preferably intravenously. For the average adult about 16 cc. should be given.