CIRCULATING IMMUNITY PRINCIPLES IN RAT-BITE FEVER.*

By YUTAKA IDO, M.D., HIROSHI ITO, M.D., HIDETSUNE WANI, M.D., AND KIKUZO OKUDA, M.D.

(From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka, Japan.)

(Received for publication, February 20, 1917.)

We owe our knowledge of the causative agent of rat-bite fever largely to the researches of the Japanese workers, Futaki and Ishiwara, and their associates. Futaki in 1915, reported finding a spirochete in preparations from the bitten area and the lymph glands of persons having rat-bite fever. Ishiwara and Ohtawara, during the same year, conducted histological studies on guinea pigs which had been experimentally infected with rat-bite fever, and found a similar spirochete. In 1916, numerous other authors in Japan took up the problem which centered particularly in the variety of types of spirochetes found in the disease in man and the experimental animals. Two main groups—long and short spirochetes—were distinguished. In May, 1916, Futaki announced on the strength of cultural studies made by him and his associates, that the two types are in all probability identical, and a little later he definitely advanced the belief that the organism is the causative agent of rat-bite fever. His work was confirmed by Kaneko and Okuda,[1] who reported on the pathological anatomy of the disease.

The short spirochete found by Futaki, and Kaneko and Okuda, coincides with respect to form and movement with that observed by Ishiwara and his associates in experimentally infected guinea pigs. These spirochetes have been identified in guinea pigs, white rats, and mice, following the intraperitoneal injection of patients' blood and emulsion of lymph gland. In the guinea pig, fever resulted. In no instance were these spirochetes found in healthy guinea pigs, white rats, or mice. They were also absent from the blood and tissues of infected animals receiving salvarsan treatment.

On the basis of the facts above cited we may say that in all probability the spirochete which has been described is the causative agent of rat-bite fever. The premises are, however, not complete, for we

* Published in *Tokyo Iji-Shuho*, September, 1916, No. 1990.

[1] Kaneko, R., and Okuda, K., A contribution to the etiology and pathology of rat-bite fever, *J. Exp. Med.*, 1917, xxvi, 363.

lack one significant proof in order to make the assertion conclusive. It is necessary to demonstrate in the serum of patients having rat-bite fever an immunity principle which is specific for the spirochetes of that disease. Up to the present time such confirmation has been lacking.

In the postmortem examination made in our clinic of a patient having rat-bite fever, the spirochetes were found located mainly in the kidneys, and not in other tissues. A similar localization of spirochetes in the kidneys is true in the convalescent stage of spirochætosis icterohæmorrhagica, and as that phenomenon, together with the disappearance of symptoms in Weil's disease, has been explained on the ground of the formation of antibodies in the blood, we may assume that a similar condition exists in rat-bite fever; *i.e.*, antibodies are present in the blood of those who recover from the disease. In order to prove this point, we obtained blood serum from three patients recovering from rat-bite fever, and conducted the experiments cited below.

The spirochetes employed by us came from guinea pigs, the animals having been infected through the bite of the rat, *Mus decumanus*. Ishiwara has affirmed that these organisms are morphologically identical with those studied by him and obtained from experimentally infected animals. We used no spirochetes taken directly from human beings.

Histories of Patients from Whom Blood Was Obtained for Animal Experiments.

Case 1.—Male, age 17, farmer. In Aug., 1915, the patient was bitten in the tip of the right little finger by a house rat. After 2 weeks, the bitten area became swollen and red, followed by swelling of right neck and axillary glands, and typical fever. Admitted to First Surgical Clinic of the University. Diagnosis, rat-bite fever. Discharged, cured, after 59 days. Blood taken on July 27, 1916.

Case 2.—Male, age 40, farmer. The patient was bitten in the upper arm by a rat, in Feb., 1916. After 20 days, the patient developed high fever, chills, swelling and sensitiveness of right axillary glands; typical exanthematous spots. Admitted to First Surgical Clinic. Treated with salvarsan. Blood taken on Aug. 4, 1916.

Case 3.—Female, age 35, wife of merchant. In May, 1916, the patient was bitten on the right upper eyelid, by a house rat. After 3 weeks, swelling and redness of bitten area; eye closed. High fever, swelling of neck glands, and exanthematous spots. Admitted to the Dermatological Clinic. Diagnosis, rat-bite fever. Blood taken on Aug. 7, 1916.

Dark-Field Preparations.

We examined by dark-field illumination a mixture of one loopful of guinea pig blood containing one to two spirochetes to an optical field and one loopful of serum from recovered cases of rat-bite fever. Control experiments were made with serum obtained from recovered cases of Weil's disease or beri-beri, and isotonic salt solution.

In the control experiments we found relatively numerous spirochetes, but we were unable to identify any actively moving organisms in the experiments made with the spirochetes and serum of rat-bite fever, although motionless or very inactive specimens were present. Here the spirochetes had been almost totally destroyed. We found only one or two specimens in a preparation.

Experiments were then undertaken to ascertain the efficacy of diluted serum. For this purpose we employed serum in isotonic salt solution, diluted two-, four-, and eightfold, and made tests similar to those which have been described. Twice diluted serum still showed the spirocheticidal and spirochetolytic properties, but with serum in a dilution of four, no such effects were observed.

It is evident from these experiments that the serum of patients convalescing from rat-bite fever contains a specific immunity principle against the spirochetes of that disease, although the immune body is relatively weak.

Pfeiffer's Phenomenon.

Pfeiffer's tests were made with the rat-bite fever serum and the blood of guinea pigs containing the spirochetes. In view of the fact that the immune bodies which develop in the blood in rat-bite fever are relatively weak, as demonstrated by the experiments described above, we employed in these tests two parts of serum to one part of

blood, in order to obtain conclusive results. The quantities used were 2 cc. of serum and 1 cc. of heart blood taken from an experimentally infected guinea pig. This mixture was injected into the peritoneal cavity of another guinea pig. After 30 minutes and again after 2 hours peritoneal fluid was drawn by puncture, and a search for spirochetes was made by dark-field illumination.

The experimental animals were also kept under observation, in order to throw further light on our results, and two series of control experiments were carried out. In the one case, we made Pfeiffer's tests with the serum of beri-beri or spirochætosis icterohæmorrhagica; in the other, we injected 1 cc. of the infected guinea pig blood but no serum. The results are shown in Tables I, II, and III.

It will be seen that no spirochetes were found in the peritoneal fluid taken 30 minutes or 2 hours after injection in Pfeiffer's tests made with the immune serum of rat-bite fever and the blood of guinea pigs containing the rat-bite fever spirochetes. On the other hand, the peritoneal fluid contained numerous, briskly moving spirochetes in the control experiments made with beri-beri or spirochætosis icterohæmorrhagica serum, as well as in the experiments where only 1 cc. of guinea pig blood, and no serum, was injected. It is clear that the serum of persons recovering from rat-bite fever has a specific spirochetolytic and spirocheticidal effect upon the organisms in question.

The blood of the experimental animals kept under observation was examined by dark-field illumination; in the case of the animals injected from Case 1 on the 12th, 20th, and 29th day after injection; of animals receiving blood from Case 2, on the 7th, 15th, and 24th day; and animals infected from Case 3, on the 5th, 13th, and 22nd day. The results in these experiments were similar to those of Pfeiffer's phenomenon tests. In the case of the guinea pig showing positive spirochetolysis, no spirochetes could be detected in the peripheral blood, while in the control animals numerous organisms were found 5 days after injection, thus proving the efficacy of the serum in protecting the guinea pig against rat-bite fever infection.

TABLE I.

Case 1.

Guinea pig No.	Injections into peritoneal cavity.		Spirochetes in peritoneal fluid.		Further course of guinea pigs. Spirochetes found in blood.		
	Patient furnishing serum. Quantity.	No. of guinea pig furnishing blood. Quantity.	After 30 min.	After 2 hrs.	12th day.	20th day.	29th day.
1	H. 2 cc. rat-bite serum, filtered.	1 cc. blood, R 27/16 V (1 spirochete in one field).	0 in one preparation.	0 in one preparation.	0 in one preparation.	0 in one preparation.	0 in one preparation.
2	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
3	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
4	" " "	1 cc. blood, R 30/16 V (1 spirochete in two fields).	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
5	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
	Control experiments.						
6	R. 2 cc. beri-beri serum.	1 cc. blood, R 32/16 V (1 spirochete in two fields).	1 in one specimen.*	1 in one specimen.	5–6 in one specimen.	2–3 in one specimen.	5–6 in one field.†
7	" " "	" " "	2–3 " " "	1–2 " " "	2–3 " " "	1–2 " " "	3–4 " " "
8	" " "	" " "	2–3 " " "	1–2 " " "	Died on 4th day, diarrhea.		
9		" " "	1 " " "	1 " " "	Killed on 4th day, diarrhea. Spirochete found in blood.		

* Cover-glass preparation, 70 optical fields, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

† Optical field, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

TABLE II.

Case 2.

Guinea pig No.	Injections into peritoneal cavity.		Spirochetes in peritoneal fluid.		Further course of guinea pigs. Spirochetes found in blood.		
	Patient furnishing serum. Quantity.	No. of guinea pig furnishing blood. Quantity.	After 30 min.	After 2 hrs.	7th day.	15th day.	24th day.
10	E. 2 cc. rat-bite serum, filtered.	1 cc. blood, R 31/16 V (more than 1 spirochete in field).	0 in one preparation.	0 in one preparation.	0 in one preparation..	0 in one preparation.	0 in one preparation.
11	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
12	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
	Control experiments.						
13	K. 2 cc. beri-beri serum.	1 cc. blood, R 34/16 V (more than 1 spirochete in field).	4 in one specimen.*	3–4 in one specimen.	1–2 in one specimen.	1 in three to four fields.†	2 in one field.
14	" " "	" " "	1–2 " " "	2–4 " " "	1 in two to three specimens.	1 in one field.	4–5 " " "
15	" " "	" " "	4 " " "	4–5 " " "	1–2 in one specimen.	1 in one to two fields.	1 in one to two fields.
16		" " "	5–6 " " "	4 " " "	1–2 " " "	1–2 in one field.	3–4 in one field.

* Cover-glass preparation, 70 optical fields, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

† Optical field, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

TABLE III.

Case 3.

Guinea pig No.	Injections into peritoneal cavity.		Spirochetes in peritoneal fluid.		Further course of guinea pigs. Spirochetes found in blood.		
	Patients furnishing serum. Quantity.	No. of guinea pig furnishing blood. Quantity.	After 30 min.	After 2 hrs.	5th day.	13th day.	22nd day.
17	G. 2 cc. rat-bite serum, filtered.	1 cc. blood, R 29/16 V (1–2 in one field).	0 in one preparation.	0 in one preparation.	0 in one preparation.	0 in one preparation.	0 in one preparation.
18	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
19	" " "	" " "	0 " " "	0 " " "	0 " " "	0 " " "	0 " " "
	Control experiments.						
20	H. 2 cc. spirochætosis icterohæmorrhagica serum, filtered.	1 cc. blood, R 30/16 V (2 in one field).	5–6 in one specimen.*	6 in one specimen.	1 in two to three specimens.	5–6 in one specimen.	3–4 in one field.†
21	" " "	" " "	7 " " "	5 " " "	" " "	2–3 in one field.	2–3 " " "
22	" " "	" " "	7–8 " " "	7 " " "	" " "	Died.	
23		1 cc. blood, R 29/16 V (1–2 in one field).	5 " " "	6 " " "	" " "	7–8 in one specimen.	1–2 in one field.

* Cover-glass preparation, 70 optical fields, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

† Optical field, Leitz oc. 3, obj. $\frac{1}{12}$ oil immersion.

The Action of Rat-Bite Fever Serum on the Spirochetes Circulating in the Blood.

We injected 1 to 3 cc. of serum from the three cases of rat-bite fever intraperitoneally, intravenously, or subcutaneously into guinea pigs. The results were wholly negative, as the spirochetes in the circulating blood were not affected. We believe, however, that the negative character of the experiments is due to the small quantity of serum employed, and that in order to obtain a decisive result it is necessary to use for injection into the experimental animals serum in quantities corresponding to the total amount of blood employed.

As the guinea pig requires a large amount of serum on account of its size, we used mice in our further experiments. The maximum body weight of a mouse is 10 gm., and hence the amount of serum needed is not large; we found that 1 cc. of serum is effective in the mouse. This quantity of the immune serum of rat-bite fever was injected intraperitoneally into two mice, and intravenously into another. In the mouse receiving the intravenous injection, we were unable to find, 30 minutes after inoculation, any spirochetes in the blood by dark-field illumination, while numerous organisms had been detected prior to the injection. In the other mice, those which had received intraperitoneal injections, the number of spirochetes 30 minutes after inoculation was greatly decreased, only one or two specimens being found in a preparation after diligent search. It must be added, however, that in the latter case an increase in the number of spirochetes in the blood took place 1 or 2 days later. In the intravenously injected mouse, a small number of spirochetes could be detected in the blood on the 3rd day following the injection.

SUMMARY.

Summarizing the results that have been cited, we have proved that the blood serum of convalescents from rat-bite fever contains antibodies which are specific against the causative agent of that disease. The serum of rat-bite fever was capable of destroying the spirochetes not only in the hanging drop preparations, but also in the peritoneal cavity of guinea pigs. The guinea pigs employed for

Pfeiffer's test always remained well. In the experimentally infected mice receiving intravenously or intraperitoneally serum equalling in quantity the amount of infected blood, the numbers of spirochetes were greatly decreased or they disappeared for a definite period. It is not yet clear how long after recovery from rat-bite fever the antibodies are effective in the blood of human beings, and further investigations are needed to elucidate this point. In our experiments we found that serum taken from Case 1 showed definite spirochetolytic and spirocheticidal properties 11 months after the onset of the disease. In Case 2, the period was 6 months, and in Case 3, 3 months following the onset of rat-bite fever.

CONCLUSIONS.

1. The serum of persons who have recovered from rat-bite fever contains an immune body which destroys the spirochetes of that disease.

2. The experiments here cited confirm the findings of Futaki and his associates, with respect to the spirochete which is identical with the organism discovered by Ishiwara and Ohtawara and advanced as the causative agent of rat-bite fever. The etiology of rat-bite fever has, therefore, been definitely established.

3. The immune body present in blood serum during the convalescent stage of rat-bite fever is not equal in efficacy to that contained in the serum of spirochætosis icterohæmorrhagica.

We take pleasure in expressing our appreciation of the direction and support which Dr. Ryokichi Inada has given to our work.