THE PROPHYLAXIS OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

By YUTAKA IDO, M.D., ROKURO HOKI, M.D., HIROSHI ITO, M.D., AND H. WANI, M.D.

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

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The prevention of Weil's disease can be undertaken on the basis of the excretion of the pathogenic cause in the urine and feces of patients, and on the routes of invasion of the spirochetes into the human body. But prophylaxis by active immunization seems to offer the surest method.

Prophylaxis by Active Immunization.

Before proceeding to the active immunization of the human body we made preliminary experiments on guinea pigs. The materia, to be inoculated (vaccine) was made in the following manner: To the liver emulsion or the pure culture (in Noguchi's culture medium as modified by Inada and Ido), which contained from 10 to 15 spirochetes in a single field,[1] carbolic acid was added in the proportion of 0.5 per cent, after which the mixture was left for 1 week in the ice box. The clear supernatant fluid was employed for injection into the peritoneal cavity of the guinea pigs three times at intervals of 7 to 9 days. The quantity introduced at the first injection was 2 to 3 cc., at the second 2 to 4 cc., and at the third 2 to 4 cc. The immediate effect of the injection was sometimes to cause feeble convulsions of the body, which disappeared completely in half an hour. The preservative may be responsible for this effect. The temperature often rose to 38–40°C. and continued there for 1 to 2 days. In some instances the guinea pigs, as a result of repeated inoculations, lost weight and appetite, and died. From 9 to 18 days after the last inoculation, 2 to 3 cc. of a liver emulsion or of the pure culture con-

[1] $\frac{1}{12}$ oil immersion, oc. 3 (Leitz), under dark-field illumination.

TABLE I.

Animal No.	Date and quantity of serum inoculated.			Injection of spirochetes.		Result.	Day of disease of control animals.	Result of Pfeiffer's test with blood serum of experimental animals.
	I	II	III	Date.	Quantity.			
1	May 26 2 cc.	June 1 2 cc.	June 9 2 cc.	June 26	Liver emulsion 2 cc.	Died on same day.		
2	"	"	"	" 24	"	Condition good until 23rd day. Killed.	Died of icterus on 5th day.	Positive.
3	May 1 2 cc.	May 24 2 cc.	May 30 2 cc.	" 17	"	Died on following day. Autopsy findings negative.		
4	June 26 2 cc.	July 4 2 cc.	July 10 4 cc.	July 16	"	Died on 8th day. No icterus. Autopsy findings negative.		
5	July 4 2 cc.	July 10 4 cc.	July 16 4 cc.	" 26	Pure culture 2 cc.	Condition good until 19th day. Killed.	Died of icterus on 5th day.	Positive.
6	"	"	"	"	"	"	"	"
7	July 8 2 cc.	July 14 4 cc.	July 22 4 cc.	Aug. 1	Pure culture 3 cc.	Died of dysentery on 14th day. Autopsy findings negative.	"	

8	July 8 2 cc.	July 14 3 cc.	July 22 4 cc.	Aug. 1	Pure culture 3 cc.	Condition good until 17th day. Killed. Autopsy findings negative.	Died of icterus on 5th day.	Positive.
9	"	"	"	"	"	"	"	
10	"	4 cc.		"	"	"	"	
11	July 10 3 cc.	July 16 4 cc.	July 22 4 cc.	"	"	"	"	
12	"	"	"	"	"	Died of dysentery on 14th day. Autopsy findings negative.	"	
13	"	"	"	"	"	Condition good until 17th day. Killed. Autopsy findings negative.	"	
14	"	"	"	"	"	"	"	Positive.
15	"	"	"	"	"	Died on 3rd day. Autopsy findings negative.	"	

taining 6 to 15 spirochetes in a field were injected into the peritoneal cavity. Table I gives the result of this experiment. 9 of the 15 animals survived for 17 days or longer. 6 died of diarrhea, 1 on the day of injection, 1 the day after, 1 on the 3rd day, 1 on the 8th, and 2 on the 14th day. The autopsy findings were negative. They showed neither icterus nor hemorrhage, and no spirochetes were found in the liver or kidneys under dark-field illumination. The livers of the guinea pigs that died on the 8th and 14th days were emulsified and injected intraperitoneally into the other guinea pigs. Of these animals only the first died of the typical disease, while the others remained healthy. That the spirochetes used for the infection were highly virulent was shown by the control animals, which, having been inoculated at the same time with an equal quantity of pure culture, succumbed after 5 days to the typical disease. Hence it was shown that guinea pigs are highly susceptible to Weil's disease and can be immunized actively by inoculations of a vaccine containing the killed spirochetes or their disintegration products.

In order to ascertain the fate of fresh virulent spirochetes injected intraperitoneally in immunized guinea pigs, we examined every hour the peritoneal fluid of the nine animals mentioned above. 30 minutes after the injection, the spirochetes were few and the majority already degenerated; after 2 hours either no specimen was found or very few; after 10 to 24 hours none were found. We killed the nine guinea pigs and examined them for immune bodies in the blood serum and for spirochetes in the liver and kidneys. The blood serum was positive by Pfeiffer's test, and no spirochetes were found in the liver or kidneys on dark-field illumination. From these experiments it is apparent that the spirochetes gradually disappear in from a few to 24 hours from the peritoneal cavity of the actively immunized guinea pigs; and yet that sometimes virulent spirochetes may still be present after 3 days in the liver and also in the kidneys.

It is, however, remarkable that the blood serum does not contain the immune bodies in demonstrable quantity, even after three inoculations of the vaccine, with Pfeiffer's test, although the guinea pig is protected from infection by virulent spirochetes. However, on injection of the fresh spirochetes the immune bodies appear in the blood in quantities demonstrable by Pfeiffer's test. We shall have something to add on this point later.

Briefly, we could show that spirochetocidal and spirochetolytic bodies develop in the blood of the guinea pig after repeated inoculations which protect the animal from an injection of fresh, virulent spirochetes. We have not yet tested the duration of this active immunity.

Passive immunization is perhaps not possible. We injected as much as 10 cc. of the immune serum into guinea pigs, which was followed after several days by inoculation of active spirochetes. All the animals developed typical infections. No protection was given. But when immune serum and spirochetes were injected at the same time, no infection took place; the guinea pigs were protected from the disease.

Prophylaxis through Protective Inoculation in Man.

We undertook the immunization of a horse through inoculation with vaccine. After demonstrating the appearance of immune bodies in the blood of the horse, we proceeded to the active immunization of man.

At first we tested the vaccine which was employed for immunizing the guinea pigs, but unsuccessfully. Later we prepared a vaccine ten times as strong as the first and with it obtained good results for the first time. Individuals subject to, but who had not had Weil's disease were inoculated subcutaneously, and tests were made for the immune bodies after a definite period.

The body temperature of each person was taken for 1 week. The blood was taken from a vein, and the serum kept in the ice box. The inoculations were made at intervals of 5 days, at the first injection 0.5 cc., at the second 1 cc., and at the third 2 cc. being given. The blood was drawn 10 days after the last injection. Pfeiffer's tests were made with the serum taken before and after the vaccine injection, and with them a control test with isotonic salt solution was made also. The results of the tests are shown in Table II.

From the table it is seen that Pfeiffer's test made with the serum taken before inoculation does not differ from the control test, while that made with the serum drawn after the inoculation shows the presence of immune bodies.

TABLE II.

Experiment No.	Animal No.	Inoculated into peritoneal cavity of guinea pigs.*		No. of spirochetes in peritoneal fluid.		Result.	Autopsy findings.	Spirochetes in liver.
		Serum.	Spirochetes.	After 30 min.	After 2 hrs.			
Control.		Isotonic salt solution 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 4th day.	+++	+++
1	1	K. G. serum before inoculation 1 cc.	"	"	1–2 in 1 f.	Died of icterus on 6th day.	+++	+++
	2	K. G. serum after inoculation 1 cc.	"	1–2 in 1 l.	1 in one specimen.	Died on 13th day. No icterus.	+	+
2	3	E.G. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 1 f.	Died of icterus on 4th day.	+++	+++
	4	E.G. serum after inoculation 1 cc.	"	2–3 in 1 l.	1 in 1 l.	Died of icterus on 8th day.	++	++
3	5	F.T. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 1 f.	Died of icterus on 6th day.	+++	+++
	6	F.T. serum after inoculation 1 cc.	"	1 in 3 f.	1 in 2 l.	Died of icterus on 10th day.	++	++
4	7	N. Z. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 2 f.	Died of icterus on 4th day.	+++	+++
	8	N. Z. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died of icterus on 12th day.	+	++
Control.		Isotonic salt solution 1 cc.	Liver emulsion (10 in 1 f.) 1 cc.	7–8 in 1 f.	6–7 in 1 f.	Died of icterus on 4th day.	+++	+++
5	9	M. S. serum before inoculation 1 cc.	"	6–8 in 1 f.	5–6 in 1 f.	Died of icterus on 4th day.	+++	+++
	10	M. S. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died of icterus on 8th day.	+++	+

Control.		Isotonic salt solution 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 3rd day.	++	+++
6	11	I. H. serum before inoculation 1 cc.	"	1 in 1 f.	2–3 in 1 l.	Died of icterus on 6th day.	+++	+++
	12	I. H. serum after inoculation 1 cc.	"	1–2 in 1 l.	1 in a few l.	Died of icterus on 9th day.	++	+
Control.		Isotonic salt solution 1 cc.	Liver emulsion (10 in 1 f.) 1 cc.	1–2 in 1 f.	1–2 in 1 f.	Died of icterus on 6th day.	+++	+++
7	13	M. K. serum before inoculation 1 cc.	"	"	"	Died of icterus on 5th day.	+++	+++
	14	M. K. serum after inoculation 1 cc.	"	2 in a few l.	1 in one specimen.	Died of icterus on 10th day.	++	++
8	15	K. S. serum before inoculation 1 cc.	"	1 in 1 f.	1 in 1 f.	Died of icterus on 6th day.	+++	+++
	16	K. S. serum after inoculation 1 cc.	"	1 in one specimen.	None in one specimen.	Died of icterus on 11th day.	++	+
9	17	A. K. serum before inoculation 1 cc.	"	1–2 in 1 f.	1 in 1 f.	Died of icterus on 5th day.	+++	+++
	18	A. K. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died on 15th day. No icterus.	+	+

*The spirochetes were always searched for with dark-field illumination ($\frac{1}{12}$ oil immersion, oc. 3, Leitz).
f. = one field.
l. = from one side to the other of the cover glass (65 to 70 fields).

In the first instance one sees that as regards Pfeiffer's test the spirochetes in the peritoneal fluid are identical with the serum taken before the inoculation and the salt solution; while the blood serum taken after the inoculation yields after 30 minutes scarcely any spirochetes, and after 2 hours merely a degenerate specimen in a preparation. The guinea pig died, however, after 13 days, without icterus; that is, 7 to 9 days later than the control animal and the animal with serum taken before the inoculation. The internal organs showed some hemorrhage and a few spirochetes in the liver. Hence the existence of immune bodies in the blood is shown, although still insufficient to prevent all infection. The ninth animal gave a similar result. The other animals did not show as high a grade of immunity as these two. The difference in survival between the immunized and the control animals amounts in several cases, respectively, to 7, 8, and 10 days, in 2 cases to 5, in 3 cases to 4, and in 1 case to 3 days. The difference of 4 days indicates the lowest limit of the incomplete immunity. If the serum contains no immune bodies, as is the case in the blood serum of normal individuals, the experimental animal succumbs to the typical disease on the same day as the control, or at the most, a day or two earlier or later. A difference of 3 days is seldom seen. If, therefore, the experimental animal dies of the typical disease 4 days later than the control animal, we may conclude that the blood serum of this animal contains a certain quantity of immune bodies. The greater the quantity of immune bodies in the blood serum, the longer is the time of incubation for the guinea pig, and finally when complete immunity is attained, the animal no longer becomes ill after injections. This phenomenon of gradually increasing immunity can readily be followed by Pfeiffer's test of the blood drawn each day after the 6th or 7th day of the disease. This experiment thus indicates that the immune bodies appear in the blood after inoculation.

The question arises as to whether or not this incomplete immunity is able to protect the organism against infection with *Spirochæta icterohæmorrhagiæ*. As we are not in a position to decide this question by the injection of the pathogenic cause directly into man, we are obliged to draw our conclusions indirectly from the immunity reaction of patients and from animal experimentation.

This state of incomplete immunity is observed in patients after the 8th to the 11th day of the disease. In this stage (the second stage according to Inada and Ido, or the icteric period according to Oguro) the experiments of infection with the blood of the patient are, as a rule, negative. The spirochetes in the blood have all been killed by the immune bodies. After the 8th or 9th day of the disease, there are likewise no, or very few degenerated spirochetes in the liver. The spirochetes in the liver have already been destroyed by the immune bodies in the incomplete state of immunity. It is not possible to demonstrate immune bodies by Pfeiffer's test within a week after the onset of the disease. The experimental animals die as rapidly as the controls. Nevertheless, one recognizes from the infection experiments conducted with the blood of patients, that a certain quantity of the immune bodies must have appeared in the blood. The intraperitoneal injection of 2 or 3 cc. of blood into the guinea pig produces the disease in typical form up to the 4th day in 100 per cent of cases (26 cases, all positive), on the 5th day in 91.6 per cent (only 1 case among 12 being negative), on the 6th day in 85.7 per cent (only 2 negative cases among 14), on the 7th day in 50 per cent (4 negative and 4 positive cases). These gradually decreasing percentages of positive results can be explained only by the appearance of the immune bodies. The almost completely negative findings in the liver on the 7th day of the disease indicate the appearance of immune bodies even inside of 1 week. Nevertheless, up to the 7th day we cannot prove the existence of immune bodies in the blood serum by Pfeiffer's test. But, we may conclude that the degree of partial immunity described above suffices to protect the organism against infection from the spirochete of Weil's disease.

By means of these animal experiments it was possible to establish the fact that three inoculations protect the guinea pig against infection with the spirochete; but notwithstanding this active immunity, it is not possible to demonstrate by Pfeiffer's test immune bodies in the blood serum of the guinea pig (Table III).

We inoculated six guinea pigs, giving each three injections of 1 cc. of vaccine after an interval of 5 days, and then injected intraperitoneally into three guinea pigs pure cultures of *Spirochæta ictero-*

TABLE III.

Animal No.	Date and quantity inoculated. I	II	III	Spirochetes injected.	Result.	Autopsy findings.	Spirochetes in liver.	Result.
1	Jan. 20 1 cc.	Jan. 25 1 cc.	Jan. 29 1 cc.	Pure culture (30 in 1 f.) 1 cc.	Died of dysentery on 19th day.	−	−	−
2	"	"	"	"	Died of dysentery on 16th day.	−	−	−
3	"	"	"	"	Died of dysentery on 18th day.	−	−	−
Control.				"	Died of icterus on 4th day.	+	+	+
1	Jan. 22 1 cc.	Jan. 27 1 cc.	Jan. 31 1 cc.	Not injected.	Killed Feb. 9 to get blood serum.			
2	"	"	"	"	"			
3	"	"	"	"	"			

Pfeiffer's test.

Animal No.	Inoculated into peritoneal cavity of guinea pigs. Serum.	Spirochetes.	No. of spirochetes in peritoneal fluid. After 30 min.	After 2 hrs.	Result.	Autopsy findings.	Spirochetes in liver.	Result.
1	V_1 serum 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 5th day.	+++	+++	−
2	V_2 serum 1 cc.	"	"	1 in 1 f.	"	+++	+++	−
3	V_3 serum 1 cc.	"	"	2 in 1 f.	Died of icterus on 4th day.	+++	+++	−
4	Isotonic salt solution 1 cc.	"	"	2–3 in 1 f.	"	+++	+++	−

hæmorrhagiæ. Three other animals were killed and their serum was tested by Pfeiffer's method. The three guinea pigs into which the pure cultures were injected died of diarrhea after 16 to 19 days, without showing the typical disease. The autopsy findings were completely negative in all. No spirochetes could be found in the organs. 4 days later, the control animal died of the typical disease. In the serum of the three guinea pigs that were killed no trace of immune bodies could be demonstrated by Pfeiffer's test. The experimental animals died of icterus and hemorrhage as soon as the control animal. From these experiments it is evident that subsequent to the inoculation, the serum contains immune bodies, but while the small number makes it impossible to prove the existence of the organism by Pfeiffer's test, it is sufficient to prohibit a proliferation of the spirochetes in the body of the guinea pig. After the injection of virulent spirochetes into the inoculated guinea pig, the immune bodies appear in the blood. From the clinical findings and the animal experiments we may conclude that a quantity of the immune bodies in the blood, which cannot be proved by Pfeiffer's test, acts prophylactically against infection with *Spirochæta icterohæmorrhagiæ.*

The secondary effects of the inoculation are scarcely noticeable in man. In the guinea pig we often observed fever following an injection, but this never occurred in man. In two cases there was slight headache and general lassitude, but these manifestations were negligible and disappeared completely in from 1 to 3 days. The chief complaint was of pains at the site of injection. One often sees local swellings and slight redness, but these disappear after 24 hours. The pains also are not severe.

It is not known how long the partial immunity continues, but it is probable that it lasts from 6 months to a year.

DISCUSSION AND SUMMARY.

We have already described briefly the portals of entry and of excretion of the pathogenic spirochetes.[2] We may mention here

[2] Inada, R., Ido, Y. Hoki, R., Kaneko, R., Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

that we have twice prevented epidemics by disinfection of the ground and the removal of the inundated water in certain places in coal mines. In one mine 19 out of 50 workmen, and in another 9 out of 30 workmen came down with Weil's disease in about 2 weeks.

We have already pointed out that the period during which the pathogenic spirochetes are excreted in the urine continues, as a rule, for 40 days, and that we must, therefore, apply disinfection for at least 40 days after the first appearance of the disease. Lately we have found that in 21 cases out of 24 the spirochetes were excreted in the urine for 40 days, in one case until the 42nd day, in one case until the 45th day, and in still another case until the 63rd day.

Another important fact concerning the prophylaxis which has been brought out is that both house and ditch rats (brown) carry virulent *Spirochæta icterohæmorrhagiæ*, the causal spirochete of Weil's disease, in their kidneys. Miyajima[3] has reported that field rats have the pathogenic organisms in their kidneys; he will report these findings in detail later. The spirochetes which he described are less virulent than ours. On his advice we have carefully examined house and ditch rats in the city and rats in the coal mines of Kyushu, where Weil's disease prevails, and found that 39.5 per cent carried highly virulent pathogenic spirochetes in their kidneys, thus confirming Miyajima's experiments. The kidneys were examined microscopically under the dark-field microscope, and in the cases in which we did not find the pathogenic spirochete, we made inoculations into guinea pigs. Thus we found *Spirochæta icterohæmorrhagiæ* microscopically in the kidneys or in the urine in 32.4 per cent, and by means of inoculation in 7 per cent, making a total of 39.5 per cent carrying the pathogenic organisms, out of a total number of 86 rats examined. In some instances, rats were made to bite guinea pigs and in two instances caused Weil's disease. Among fifty-five patients in our clinic, twelve were cooks; and in Europe many cases arise among butchers—indicating the relation of the disease to rats. Moreover, during the present year we observed two patients who acquired Weil's disease, one in 1 week, the other 8 to 9 days after they had been bitten by rats. These facts point to a relation be-

[3] Reported at the April, 1916, meeting of the Fellows of the Kitasato Institute for Infectious Diseases.

tween Weil's disease and rats. The infection is transmitted probably from rats to man by means of the urine of the rats, directly or indirectly. On the injection of 0.1 gm. of rat urine which contains *Spirochæta icterohæmorrhagiæ* into the peritoneal cavity of guinea pigs, the infection arises, while the injection of the liver or the blood of the rats into guinea pigs does not produce the typical disease.

The finding that the kidneys of rats contain the pathogenic organisms of the disease is important from the point of view of prophylaxis. The large number of rats in the trenches of the European battle-fields suggests the possibility that many cases of Weil's disease may arise. We shall report on this point in more detail later.