

## THE SERUM TREATMENT OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

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Although Weil's disease has been recognized since antiquity and its mortality has been high, treatment up to the present time has been purely symptomatic, as the causative agent was not known. But with the discovery of the cause, we have now entered upon the study of the specific therapy. In the course of their discovery of the causative agent, Inada and Ido<sup>1</sup> found in the blood serum obtained from recovered cases of the disease an immune body which acted as a solvent and destroyed the spirochetes contained in the blood and the liver of the guinea pigs under experimentation; on the basis of this work they advanced the hypothesis that serum therapy should be effective in Weil's disease.

In his experimental investigation Ido obtained remarkable results with convalescent serum and active serum from immunized goats. He demonstrated that the immune serum, if injected prior to the appearance of icterus, is capable of inhibiting the disease in all cases. This work forms the basis of the serum therapy of Weil's disease in man. On the other hand, experiments undertaken with salvarsan, as already indicated elsewhere,<sup>2</sup> were not followed by comparable results. For these reasons we have been led to employ antiserum in the treatment of Weil's disease since August, 1915; and we were in a position to report the results of our work as early as October, 1915.

<sup>1</sup> Inada and Ido, A Report on the Discovery of the Causal Organism (a New Species of Spirochæta) of Weil's Disease, *Tokyo Ijishinshi*, 1915, No. 1908.

<sup>2</sup> Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., The Etiology, Mode of Infection, and Specific Therapy of Weil's Disease (Spirochætosis Ictero-hæmorrhagica), *J. Exp. Med.*, 1916, xxiii, 377.

Up to the present time serum therapy has not been widely employed in the spirochetal diseases. Since the serum therapy of readily relapsing spirilloses leads to the formation of a serum-fast strain, it has been claimed that salvarsan would be more suitable for treatment in these cases. But in Weil's disease a serum-fast strain does not occur. Spirochetolytic and spirochetocidal immune bodies, however, develop in the blood, and the immunity lasts for several years, the antibodies having in one case been unmistakably detected 8 years after recovery. Relapse apparently does not occur in Weil's disease. We have never found a case of it, although Fiedler recognizes it. Inada and Ido have given the term "after fever" to the fever which occurs on the 14th or 15th day from the onset of the disease, sometimes on the 13th to the 16th day, less frequently on the 12th to the 17th, and which continues for several days, often from 7 to 10. But this cannot be considered a relapse in the real sense of the word, because at this stage of the disease we do not find the spirochetes in the blood, or in any organs but the kidneys.

We employed first serum from recovered cases and later serum obtained from actively immunized horses. As human convalescent serum is not procurable in large quantities, a horse was immunized with *Spirochæta icterohæmorrhagiæ*. The goat also can be readily immunized, but is too small an animal to yield large amounts of serum. At the present time we possess two actively immune horses, which are able to take an injection of 800 cc. of pure culture, containing 30 spirochetes per field, under dark-field illumination ( $\frac{1}{2}$  oil immersion, No. 3 ocular, Leitz).

The serum which we now have is far more potent than that obtained at the beginning. 0.01 cc. of the present serum injected simultaneously with a pure culture into the peritoneal cavity protects a guinea pig weighing 200 gm. against infection from 1 cc. of pure culture (10 spirochetes per field). In the treatment of the patients considered here, the serum was of weaker concentration, as much as 0.5 cc. of the serum being required to protect a guinea pig against infection from a similar dose of culture. Inasmuch as our most potent serum was obtained only toward the end of 1915, the opportunity to test its action did not arise, as we have had no cases of Weil's disease since that time.

To the serum obtained from horses, 0.5 per cent carbolic acid was added and it was kept in the ice box for 1 month. It was then tested for tetanus toxin and carbolic acid content, and also by cultures, to establish sterility. We employed serum taken on July 12, August 28, September 19, and October 11 from Horse 1. 35 patients in all received the treatment; 23 in the clinic and 12 cases occurring in a coal mine. Of these patients, 6 were treated with human convalescent serum, 2 with both horse and human serum, and the remaining 27 patients with horse serum alone. As the titer of the horse serum about equalled that of the human serum, the cases treated will be discussed jointly.

*Dose of Serum Injected.*—The initial daily dose for 3 days was 10 cc., but our experience derived from a number of cases shows that this dosage is ineffectual. For that reason we employed gradually larger doses, until finally we injected 60 cc. in 24 hours. This quantity of serum was given in three amounts, with intervals of from 5 to 6 hours. Of late, we have made intravenous injections with good results. It was feared in the beginning that the toxin liberated by lysis of the spirochetes would prove harmful to the organism, but this fear proved to be unfounded.

The day on which serum treatment was instituted is shown in Table I.

TABLE I.

Day of illness.	No. of cases.
2	4
3	4
4	5
5	6
6	8
7	3
8	5

In 24 of the cases icterus was present at the beginning of the serum treatment; 11 cases showed no icterus when the treatment was begun; and in 5 there was no icterus during the course of the disease. As the serum, according to our experience, is mainly spirochetolytic and spirochetocidal in action, and its antitoxic effect has not yet been

demonstrated, it is recommended that it should be employed as early as possible after the onset of the disease,—if possible, before the 5th day of illness, when there are probably many spirochetes present in the organs. The distribution from the point of intensity of the disease was as follows: 17 severe cases, 17 moderately severe, and 1 mild case.

The criteria employed to determine the result of the serum therapy were:

1. The rate of mortality.
2. The influence of the serum upon the spirochetes in the circulating blood.
3. The effect of the serum on an earlier development of antibodies.
4. The number of spirochetes found in the organs after death, when serum treatment had been employed.
5. The influence upon the symptoms and the progress of the disease.

Of these criteria, special emphasis has been placed upon Nos. 2 and 3, inasmuch as they afford definite objective phenomena for judging the effect of the serum treatment, and are valid even if the number of patients is small, while for the other criteria a large number of observations is required. The difficulty of determining the effect upon the symptoms and the course of the disease in individual cases increases with an early use of the serum, as in that case it is not easy to decide whether the slight illness is attributable to the serum, or whether the disease as such was of a mild character. The prognosis in most cases is determined at the height of illness, or on the 7th or 8th day from the onset.

#### *Mortality in Weil's Disease.*

Of the 35 patients who received the serum treatment, 7 died. Of this number, 2 showed complications, 1 developed suppurative meningitis on the 27th day of the disease, and another patient who had a suppurative process died after 2 months. This leaves only 5 cases in which the cause of death is attributable to Weil's disease itself rather than to a complication. Of these, one patient, who was admitted in a moribund condition and died on the following day, has not been included. Omitting these cases, the mortality was found to be 11.4 per cent. If we exclude also the 12 patients in the coal mine and include only the 23 cases treated at the clinic, the mortality is 17.3 per cent.

According to Oguro, the mortality of Weil's disease in Japan is 40 per cent, according to Nishi, 48 per cent; and according to Inada, 30.6 per cent. As we are not in possession of definite data regarding the death rate among the patients in the coal mine, we have excluded these cases and have compared only the mortality of patients in our clinic, treated with serum or otherwise, with the general statistics. The mortality in our clinic up to last year was 30.6 per cent. With the beginning of the serum therapy, it will be seen that the mortality is reduced almost to two-thirds. It must, however, be stated that we have, during the past year and since the discovery of the causative agent, accepted a relatively large number of patients, and among these there were probably a number who had the disease in a mild form.

In view of the reduction in the mortality rate, at the moment, we shall limit our claim to the statement that the administration of the serum has a marked effect in Weil's disease, and we shall await the results obtained from a large number of cases before making a more positive assertion.

*The Behavior of Spirochæta icterohæmorrhagiæ in the Circulating Blood after Serum Injection.*

We desire to present in brief form the result of infection experiments carried out with the blood of patients who received no serum treatment. Table II shows the result of this investigation which comprises 68 cases, arranged according to the day of illness.

TABLE II.  
*Infection Experiments with Blood from Weil's Disease.*

Day of illness.	No. of cases.	Results.		Per cent of positive results.
		Negative.	Positive.	
Up to 4	26		26	100.0
5	12	1	11	91.6
6	14	2	12	85.7
7	8	4	4	50.0
8	4	4	0	0
9	1		1	100.0
12	1	1		0
18	1	1		0
19	1	1		0

It will be seen that in all cases up to the 4th day of illness, the result was positive; of the experiments undertaken on the 5th day 91.6 per cent were positive; on the 6th day, 85.7 per cent; on the 7th, 50 per cent. From the 8th day on, of blood taken from 8 patients, only one, that taken on the 9th day, proved to be positive.

In order to determine whether the dose of serum employed is capable of killing and dissolving the spirochetes in the circulating blood of the patient, blood was taken, before the injection of serum, from the arm vein of the patient and injected intraperitoneally into guinea pigs; and, similarly, another sample of blood was drawn after the injection of the serum. If, then, the guinea pig receiving the blood drawn before the injection of serum became typically ill, while that receiving the blood drawn after serum had been injected into the patient remained well, the last drawing of blood having been made on the 4th day of the disease, we may conclude that, in the latter case, the spirochetes in the circulating blood had been destroyed by the serum. When the blood is taken on the 5th or 6th day, the determination is somewhat more difficult, inasmuch as blood injected on the 5th day gave negative results in 8.4 per cent, and that drawn on the 6th day was negative in 14.3 per cent of the cases. It is possible to estimate the spirochetocidal action of the serum from the difference in the percentages of infectability of the injected blood (Table III).

TABLE III.

*Infection Experiments with Blood Drawn from Patients with Weil's Disease before and after the Injection of Serum into the Patient.*

No.	Day of illness on which serum treatment was given.	Results.		Quantity of serum injected.	Time between serum injection and drawing of blood.	Day of disease after serum treatment.	Positive results.	
		Before serum injection.	After serum injection.				With serum treatment.	Without serum treatment.
				cc.	hrs.		per cent	per cent
1	2	+	+	10	30	3		
2	2	+	-	40	6	3	25.0	100.0
3	2	+	-	60	24	4		
4	2	+	-	40	24	4		
5	2	+	-	60	24	5		
6	3	+	+	20	24	5		
7	3	+	±	60	24	5		
8	3	+	-	60	24	5		
9	4	+	+	20	5	5	50.0	100.0
10	4	+	+	10	7	5		
11	4	+	-	80	12	5		
12	5	+	-	20	5	5		
13	3	+	±	40	4th day.	6		
14	4	+	-	35	22	6		
15	5	+	-	40	13	6		
16	5	+	+	10	7	6		
17	5	+	+	10	24	6	71.4	77.7
18	5	-	-	60	17	6		
19	5	+	+	60	12	6		
20	6	+	+	10	3	6		
21	6	-	-	10	7	6		
22	6	+	-	10	12	7		
23	6	+	±	40	15	7	33.3	100.0
24	6	+	-	80	7	7		
25	7			60				
26	8			60			0	0
27	8	-	-	10	7	8		

Table III shows that in four cases in which human blood not subjected to serum treatment was capable of infecting guinea pigs, when drawn on the 4th day of the disease after serum treatment had been given, the infection was positive in only a single case. The

ratio of infection of the guinea pigs with blood drawn after the administration of serum is, therefore, 25 per cent. If we compare this figure with the percentages of infection (100 per cent) found in guinea pigs receiving blood taken before serum treatment, a vast difference is apparent. This difference in results justifies us fully in concluding that the blood taken from patients after they have received serum treatment is no longer capable of infecting guinea pigs. This means either that the serum has destroyed the spirochetes in the circulating blood, or that the spirochetes have lost their virulence and are no longer capable of producing an infection. The positive result in a single case is to be attributed to the insufficient dosage of the serum, only 10 cc. having been injected in the case in question. On the 5th day the percentage of guinea pigs infected after serum treatment was 50 per cent; without serum treatment, 100 per cent. The positive result in 3 cases is also due to the insufficient quantity of serum administered, as only from 10 to 20 cc. had been injected. In one case the infection experiment proved negative even when 20 cc. of the serum had been administered.

On the 6th day the percentage of illness of the guinea pigs, subsequent to serum treatment, was 71.4; without serum treatment, 77.7. Among the patients there was one who received 60 cc. of the serum, and whose blood was still capable of infecting guinea pigs after a period of 20 hours. It is difficult to say why the spirochetes in the blood were not destroyed by the serum.

On the 7th day of illness, 33.3 per cent of guinea pigs infected with blood taken after serum treatment became ill, and 100 per cent without serum treatment. Here we have the blood of a patient which caused an infection in one guinea pig, while another animal remained well. This case has been included among the positive results.

From these results we may conclude that the horse serum which we have prepared, administered in suitable doses, is capable of destroying completely the spirochetes contained in the circulating blood, by the injection of 40 to 60 cc. of serum in from 5 to 6 and up to 24 hours. A quantity of 10 to 20 cc. of the serum did not suffice to kill the spirochetes.

*The Influence of the Serum Treatment upon the Appearance of Antibodies in the Blood.*

As we have stated elsewhere,<sup>3</sup> the antibodies in Weil's disease are not demonstrable by Pfeiffer's method in the blood serum of patients inside of 1 week from the onset of illness. Beginning with the 8th or the 11th day, antibodies make their appearance in the blood, although they are at first weak; by the 12th or the 13th day they are fully active. They are rarely active as early as the 8th day. The time of development of the antibodies in the blood seems to be an individual characteristic, and differs according to the severity of the disease. We have designated as incomplete, with respect to antibody formation, those cases in which the infected animals (tested by Pfeiffer's method) outlived the control animals by 4 days or longer. A difference of 3 days in the life of the experimental and the control animals should be regarded as an indication of incomplete immunity, but we have taken 4 days as a conservative limit.

TABLE IV.

*The Period of Development of Antibodies in the Blood of Patients with Weil's Disease.*

Day of illness.	No serum treatment.				Serum treatment.			
	Complete development.	Incomplete development.	Negative.	Total.	Complete development.	Incomplete development.	Negative.	Total.
6			3	3	1	1	7	9
7			5	5		2	3	5
8	2	2	6	10	3	8	7	18
9		3	4	7	3	1	2	6
10		1	2	3	5	5	2	12
11	1		2	3		1	1	2
12	3		1	4	3			3
13	43			43	7			7

One patient treated with the serum showed complete development of antibodies in the blood on the 6th day of illness. This phenomenon never occurs with the blood of patients who have not received the serum. As shown in Table IV, in a large percentage of the patients

<sup>3</sup> Ido, Y., Hoki, R., Ito, H., Wani, H., *The Prophylaxis of Weil's Disease (Spirochætosus Icterohæmorrhagica)*, *J. Exp. Med.*, 1916, xxiv, 471.

receiving serum treatment complete development of antibodies could be demonstrated as early as the 9th or 10th day, while this condition appeared only on the 11th or 12th day when no serum had been administered. If we also include the cases of incomplete development of antibodies, we are justified in concluding that when serum treatment is given, the antibodies make their appearance in the blood earlier than in those cases where no serum is administered. As far as the dosage of serum in relation to the development of antibodies is concerned, this is difficult to estimate, for the intensity of the disease and the individual circumstances must always be taken into account, but speaking generally it may be said that development proceeds more rapidly with a large dose of serum.

*The Influence of Serum Treatment upon the Spirochetes in the Body.*

As Kaneko and Okuda<sup>4</sup> have discussed this point in detail, we shall present only a brief outline here. It may be said, in general, that the number of spirochetes found at autopsy in the bodies of patients who had received serum treatment is much less than that found in patients ill for an equal period of time who received no serum; and, moreover, the spirochetes in the first instance show a high degree of degeneration. This statement is particularly true of the kidneys and the cardiac muscles.

*The Influence of Serum Treatment upon the Symptoms and the Course of the Disease.*

Whether or not the serum has an appreciable effect upon the fever, icterus, and hemorrhagic diatheses can be determined only by more extensive experience. Up to the present time, we have observed no significant effect, except that with serum treatment the hemorrhages are less frequent than without serum treatment. The "after fever," on the other hand, seems to occur more frequently when serum has been given. Serum therapy has no definite effect upon the excretion of spirochetes in the urine, although the period of excretion of the

<sup>4</sup> Kaneko and Okuda, The Pathological and Anatomical State of the Lymph Glands in the Early Stage, and the Distribution of the Spirochæta in Them, *Tokyo Ijishinshi*, 1915, No. 1940-1945.

living spirochetes seems to be shorter where large doses of serum have been employed.

#### *Secondary Manifestations.*

We have observed few secondary effects. Following the injection of serum, no fever occurred. In one case only was there a complaint of headache, in another of tinnitus, and in still another there appeared an exanthem at the site of injection. These symptoms which were all mild in character disappeared completely after 1 or 2 days. Frequently complaint was made of slight pains at the site of injection, but these also disappeared on the following day.

#### *Spirochetocidal and Spirochetolytic Action of Serum.*

We have recently observed a case in which we were able to demonstrate experimentally upon guinea pigs in a conclusive manner the spirochetocidal and spirochetolytic action of serum from Weil's disease. The patient was a man of 65, who was taken ill suddenly on May 1. He was admitted to the clinic on the 4th day of the disease. A large number of *Spirochæta icterohæmorrhagiæ* were found in the blood under dark-field illumination; *i.e.*, 14 to 16 on a cover slip (60 to 70 optical fields). In no other case have we found the spirochetes so numerous, for as a rule the organisms are not readily found in fresh blood preparations. The number of spirochetes found before and after serum injection was as follows:

May 4, 2.00 p.m. Admitted to clinic, 4th day of illness. Spirochetes in the blood, 14 to 16 per field, as described above.

3.00 p.m. 17 cc. of serum, very active (from Feb. 26), subcutaneously.

6.00 p.m. Spirochetes 14 to 16.

7.00 p.m. Spirochetes 10 to 16.

8.00 p.m. 20 cc. of serum, intravenously.

10.00 p.m. No spirochetes in 2 preparations.

12.00 m. No spirochetes in 3 preparations.

1.30 a.m. No spirochetes in 1 preparation.

At 1.00 a.m. we injected 2 cc. of the blood of the patient into the peritoneal cavity of a guinea pig. Up to the time of writing, May 14, the animal has remained well. At 8.30 a.m., 3 cc. of the blood were injected into the peritoneal cavity of another guinea pig. This animal also has not become ill.

2 hours after intravenous injection, we were unable to find any spirochetes. This result in the experimental animal is in entire accord with the spirochetocidal and spirochetolytic action of the serum observed in human beings.

SUMMARY.

Horses immunized with cultures of *Spirochæta icterohæmorrhagia* yield an immune serum having therapeutic properties.

With rare exceptions the serum destroys completely the spirochetes contained in the circulating blood.

The development of antibodies is promoted by the serum injections.

The number of spirochetes in the organs is reduced by the treatment.

Secondary manifestations due to the serum are slight and disappear promptly.

The ultimate effects of the serum treatment on the symptoms and final outcome of the disease have still to be determined.