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ETIOLOGY OF YELLOW FEVER.

XI. SERUM TREATMENT OF ANIMALS INFECTED WITH *LEPTOSPIRA* *ICTEROIDES*.

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The high potency attained by a polyvalent immune serum for *Leptospira icteroides* derived from the horse, as revealed in previous experiments on guinea pigs,¹ indicated the possibility that such a serum might be advantageously employed in the treatment of patients suffering from an infection with that organism. In order to ascertain whether or not the serum can exert a beneficial influence upon the course of the infection, several series of experiments were planned in which the guinea pigs were first inoculated with multiple minimal lethal doses of *Leptospira icteroides* culture and then treated with the immune serum at varying intervals afterwards.

Emphasis has been laid upon the fact that guinea pigs vary considerably in their susceptibility to *Leptospira icteroides*,² and instances have been cited¹ in which some animals survived after the injection of a large amount of culture, while some succumbed to smaller amounts. Irregularities of this nature were to be anticipated in the present series of experiments, but this source of error was eliminated as much as possible by using the serum in several graduated doses on a corresponding number of animals for a number of days in succession. In some of the earlier series tests were rendered unsatisfactory by the use of a culture which, from unknown causes, failed to kill the control animals, notwithstanding the fact that the same culture had been highly virulent when tested a fortnight previously. In another series the virulence of the culture employed was such that the control

¹ Noguchi, H., *J. Exp. Med.*, 1920, xxxi, 135.

² Noguchi, H., *J. Exp. Med.*, 1919, xxix, 585.

guinea pigs died within 5 or 6 days after the injection of the culture, and the time for treatment was comparatively brief.

The serum was injected intraperitoneally in amounts of 0.001, 0.01, 0.1, and 1 cc. at intervals of 1, 24, 48, 72, and 96 hours, and even 5, 6, and 7 days, if the animals still lived, after the inoculation of the culture. In the earlier experiments two injections a day of each dose were given (10 a.m. and 4 p.m.), but this practice was soon abandoned in favor of a single daily injection.

In determining the effect of the immune serum upon the infection, the temperature and other characteristic clinical symptoms were noted, and the extent of the lesions was ascertained by killing the surviving animals at a later period, when they were regarded as convalescent. The lesions chiefly considered, although not a wholly reliable index of the severity of the infection, were the hemorrhagic foci in the lungs.

In a series of therapeutic experiments in which the control guinea pigs died within 5 to 6 days, a culture of Strain 5 of *Leptospira icteroides* being employed, it was found that when injections of the immune serum were begun within 24 hours from the time of the experimental infection (intraperitoneal), no symptoms or lesions developed, provided the animal had received more than 0.001 cc. of the serum. If the treatment was begun 48 hours after infection, it was necessary to inject more than 0.01 cc. in order to prevent their development. At the end of 72 hours, when some of the guinea pigs had begun to show a rise of temperature, more than 0.1 cc. of the serum was necessary to check the progress of the infection, although some animals treated with 0.01 cc. also recovered. After 96 hours, when most of the animals had a high temperature, and some had begun to show a trace of jaundice, the injection of more than 0.01 cc. had a marked influence. The temperature came down to 100–102°F. by the following morning, and no jaundice afterwards appeared in these animals, which recovered within a week. A few guinea pigs, which were slightly icteric at the time of injection of the serum, became more deeply jaundiced on the following day and remained so for a few days, when they began to convalesce. No guinea pig treated within 96 hours with more than 0.01 cc. of serum died.

At the end of 5 days most of the animals which had not been treated with the serum showed jaundice and a decline in temperature, signs of approaching death within 24 hours. A few animals died in the afternoon of that day. To several of these critically ill guinea pigs 0.1 to 1 cc. of the serum was given twice, at 10 a.m. and at 4 p.m., but none of them seemed to be influenced by the injection. Some died on the same day, others on the following day, showing all the typical symptoms. At the end of 6 days several animals which still remained alive without the serum were treated with two doses of 1 cc., but none was saved from death on the same day.

It was found from this series of experiments that the progress of jaundice, after it had existed for 24 hours, could not be checked by the injection of the immune serum.

All the surviving animals were later killed for examination of the lung lesions. The animals treated with a sufficient amount (0.001 cc. or more) within 24 hours showed no lesions, or at most only a few hemorrhagic spots in the lungs. On the other hand, fairly numerous old hemorrhagic foci were found in the lungs of those which had been treated at the end of 48 hours, indicating an infection aborted through the action of the serum. The lung lesions were decidedly more diffuse and numerous in the guinea pigs which received the serum at the end of 72 and 96 hours, although none succumbed to the infection. In the animals which died following treatment with the serum later than 5 days after infection the symptoms and lesions were typical of the infection and indistinguishable from those in the control animals which died without receiving any serum.

In Table I is given another series of experiments carried out with Strain 6 of *Leptospira icteroides*. This culture killed control animals in doses of 0.001, 0.01, and 0.1 cc. within 13, 10, and 7 days respectively, although one guinea pig inoculated with 1 cc. of the culture escaped death after a severe infection. The amount of culture employed throughout the series was 0.5 cc. and was given intraperitoneally. The injections of serum were begun 1 hour after the inoculation and repeated daily for 7 days, in doses of 0.01, 0.1, and 1 cc.

As the table shows, no infection, so far as external manifestations are concerned, took place in any of the guinea pigs injected with the

TABLE I.
Effect of Polyvalent Immune Serum on Experimental Infection with *Leptospira icteroides*.

June 13, 1919. Thirty-seven guinea pigs were inoculated intraperitoneally, each with 0.5 cc. of Strain 6 of *Leptospira icteroides* culture, representing at least 500 minimal lethal doses. Graduated quantities (0.01, 0.1, and 1 cc.) of Immune Horse Serum 2 were given to the inoculated guinea pigs at intervals of 1, 24, 48, 72, and 96 hours, etc. The object of the experiment was to ascertain the minimal amount of the immune serum which prevents a fatal infection and the maximal time within which the inoculated animals may still be saved by the injection of the serum.

Guinea pig No.	Time elapsed after inoculation.		Amount of serum injected.	Conditions preceding injection of immune serum.	Subsequent course of infection (after injection of immune serum).	Remarks.*
	Hrs.	Cc.				
x194a	1	0.01	No noticeable effects from inoculation.	No symptoms.	No lesions found.	
x194b	1	0.1	"	"	"	
x195a	1	1.0	"	"	"	
x195b	24	0.01	"	"	Several old hemorrhages in lungs.	
x196a	24	0.1	"	"	A few minute foci of old hemorrhages in lungs.	
x196b	24	1.0	"	"	No lesions found.	
x197a	48	0.01	"	"	Rather diffuse old hemorrhages in lungs.	
x197b	48	0.1	"	"	No lesions found.	
x198a	48	1.0	"	"	A few old hemorrhagic foci in lungs.	
x198b	72	0.01	"	"	Several old hemorrhagic foci in lungs.	
x199a	72	0.1	"	"	No lesions found.	
x199b	72	1.0	"	"	A few old hemorrhagic spots in lungs.	

x ₁ 100a	96	0.01	Temperature 104°F. in a.m. but animal not apparently sick; no jaundice. 1st day of disease (4 day incubation period). Serum given in p.m.	Temperature 105.5°F. after injection of serum. Following a.m. 102.5°; 101.5° in p.m. No further symptoms.	A few old hemorrhagic foci in lungs.
x ₁ 100b	96	0.1	Temperature 104.5° in a.m.; no other symptoms. Serum given in p.m.	Temperature 104.5° in p.m.; normal next day. Animal well thereafter.	Numerous old hemorrhagic foci in lungs.
x ₁ 101a	96	1.0	Animal remained well (temperature 102°) after injection of serum.	No change in temperature (102.5°). Animal remained well throughout experiment.	No lesions found.
x ₁ 101b	120	0.01	Had had high temperature (104°, 103°) 2 preceding afternoons. Temperature not high in a.m. when serum was given (101.5°).	Temperature 103.5° in p.m.; animal, however, seemed well. No further symptoms. Temperature returned to normal following day. Survived.	“ “ (natural refractoriness suspected).
x ₁ 102a	120	0.1	Fever since preceding day (103.5–104°). Temperature in a.m. on serum injection 103°; rose to 104° in p.m.	Temperature next day 103.5° in a.m. and 104.5° in p.m., but on following a.m. 101.5°. Afternoon rise to 104° for 2 more days, then return to normal. Animal survived.	Numerous pale hemorrhagic foci in lungs.
x ₁ 102b	120	1.0	Temperature 104° in a.m. and 105.5° in p.m. of day before. 104.5° in a.m. of day serum was given in p.m. Jaundice not distinct.	Temperature remained 104.5° in p.m. 102° next a.m. and 101° in p.m. No further febrile or other symptoms. No jaundice developed. Survived.	Pale hemorrhagic foci in lungs.

* All the guinea pigs surviving were killed on July 4, 1919 (21 days after infection) for examination.

TABLE I—Continued.

Guinea pig No.	Time elapsed after inoculation.		Amount of serum injected.	Conditions preceding injection of immune serum.	Subsequent course of infection (after injection of immune serum).	Remarks.*
	days	cc.				
x103a	6	0.01	Fever for 3 preceding days (103.5°, 104°, 104.5°). 102° in a.m. of serum injection. Distinct jaundice; animal very weak, bordering on collapse.	Temperature rose to 104° in p.m. after serum injection. Animal gradually became worse, dying 9 days after infection.	<i>Autopsy.</i> —Extreme jaundice, and general hemorrhage, especially in lungs.	
x103b	6	0.1	Fever for 3 preceding days (103°, 104.5°, 104.5°). Temperature 104° in a.m. of injection. Slight trace of jaundice.	Temperature 104.5° in p.m. after injection of serum. Fever disappeared next day. Animal well throughout remainder of experiment. Survived.	Extensive foci of old hemorrhages in lungs.	
x104a	6	1.0	Fever for 3 preceding days (104.5°, 106°, 105°). 103° in a.m. of serum injection. Mild jaundice.	Temperature same p.m. 104.5°, but only 101° next day. Jaundice persisted 3 days longer, then faded. Survived.	Extensive foci of old hemorrhages in lungs.	
x104b	7	0.01	Fever for 2 preceding days (105°, 102.5°). Temperature 104° on day of injection. Jaundice slight.	Temperature 104° after serum injection, but 102° next 3 days, returning slowly to normal. Jaundice disappeared in few days.	Rather numerous old hemorrhagic lesions in lungs.	
x106a	7	0.1	Fever 3 preceding days (106°, 104.5°, 103.5°). Temperature 102.5° in a.m. of serum injection. Distinct jaundice. Serum given in p.m.	Temperature 103.5° same p.m., 102.5° next a.m., 103.5° p.m., 102° next a.m., 101° p.m. Jaundice persisted 3 days, then faded. Survived.	Very marked hemorrhagic lesions in lungs.	

x ₁ 107a	7	1.0	Fever 3 preceding days (105°, 104.5°, 103°). Temperature 102° in a.m. of serum injection. Marked jaundice. Serum given in p.m.	Temperature 103.5° in p.m.; 101-101.5° on days following. Jaundice increased for 2 days after injection of serum, disappearing 5 days later. Survived.	Hemorrhagic foci in lungs less marked than in preceding animal.
x ₁ 107b	7	1.0	High temperature for 4 afternoons preceding (104°, 104°, 103°, 104°), but less in mornings (102.5°, 104°, 102°, 102°). Temperature 102.5° in a.m. of serum injection. Jaundice intense for 2 days. Serum given in p.m.	Temperature 103.5° same p.m.; 100° next a.m. Jaundice and collapse. Died 8 days after inoculation.	<i>Autopsy.</i> —Typical lesions.

Controls.

Guinea pig No.	Amount of culture.	Course of infection.	Remarks.*
x ₁ 192a (control).	0.001 <i>cc.</i>	Incubation of 9 days, followed by fever for 4 days (104°, 102°, 105.5°, 103°F.). Jaundice noticed on 3rd day of disease; increased in intensity for 3 days following. Death occurred, with temperature 98°, 13 days after inoculation.	<i>Autopsy.</i> —Typical lesions.
x ₁ 192b (control).	0.01	Incubation of 4 days, followed by period of fever for 5 days. Jaundice developed in 7 days and became extremely intense within 24 hrs., remaining so until death, which occurred on 10th day after inoculation, with temperature of 96.5°.	" "
x ₁ 193a (control).	0.1	Incubation period 4 days, followed by fever for 48 hrs. (104.5°, 103.5°), then collapse, with temperature 99° on 8th day. Jaundice appeared on day before death, which occurred 7 days after inoculation.	" "
x ₁ 193b (control).	1.0	Temperature 103.5° on 4th day after inoculation. Fluctuated for 48 hrs. longer, but was at no time higher. Jaundice appeared on 6th day, increased in intensity rapidly during next 48 hrs., then receded, having disappeared 4 days later. Animal survived.	Killed. Extensive hemorrhagic lesions in lungs.

serum within a period of 72 hours from the time of inoculation with the culture. All remained well.

At the end of 96 hours most of the animals had a temperature of 103–104.5°F. in the morning. The injections of the serum were made in the afternoon. The temperature remained high after the injection but dropped gradually the following day, and the animals recovered rapidly.

At the end of 5 days most of the animals had had a high fever for 2 days. The serum was given in the afternoon. Two of the three guinea pigs treated began to improve the next day, one had a high temperature for a day longer, but all eventually returned to the normal condition.

At the end of 6 days the animals had begun to show more or less jaundice and had had fever for 3 days. One guinea pig which received 0.01 cc. of the serum died 9 days from the time of infection, while those which received 0.1 and 1 cc. recovered.

Experiments were made at the end of 7 days, when the animals had had fever for about 3 days, and a slight decline had begun in the morning. In some there had been jaundice for 48 hours. The serum was injected in the afternoon. The temperature the following morning was quite high in most animals, and in some it lasted for 2 to 3 days, while in others it went down rapidly. Jaundice increased in intensity in some, but gradually disappeared within several days. All recovered except one, which was near collapse when 1 cc. of serum was injected and which died the next day.

Of the surviving guinea pigs, no lesions were found in the lungs of those treated with the serum within 1 hour; and in each group of three guinea pigs treated 24, 48, 72, or even as late as 96 hours (during the incubation period) there was one animal in which no lesion was present. In others, irrespective of the amount of serum given, there were a certain number of hemorrhagic spots in the lungs, even when treated with the serum within 24 hours. In one instance there was no lung lesion in a guinea pig treated with 0.01 cc. after 5 days. These irregularities are explained by the existence of a considerable variation among individual animals in their susceptibility to *icteroides* infection.

On the whole, the results obtained in this series admit of only one interpretation; namely, that the immune serum, when injected during the period of incubation, prevents further development of the infection, and that when used in the early stage of the disease it is capable of preventing a fatal termination of the infection. On the other hand, when guinea pigs are inoculated with a highly virulent culture, the injection of the serum at a period of the disease when jaundice has existed for some time and the animal is nearing collapse seems to have no benefit. Undoubtedly the virulence of the strain employed for the experiments has considerable bearing upon the efficacy with which the immune serum may be used in the late stage of the disease. The less virulent the culture, the greater are the chances of benefit from the injection of the serum after the clinical symptoms have manifested themselves, as shown by the favorable results obtained in the last series of experiments just recorded. The fact must be emphasized, however, that even with a less virulent strain the serum has no beneficial effect when given to a guinea pig which is in a condition bordering on collapse (fall of temperature, with intense jaundice).

SUMMARY.

The use of a polyvalent immune serum of high potency in the treatment of an experimental infection of guinea pigs with *Leptospira icteroides* was found to be of definite advantage in checking the progress of the infection. When administered during the period of incubation the serum was found capable of completely preventing the development of the disease, although on subsequent examination hemorrhagic lesions of greater or less number and extent were found in the lungs of the guinea pigs which survived. Moreover, the serum modified the course of the disease and when used in the early stages of infection prevented a fatal outcome. Employed at a later stage, however, when jaundice and nephritis had been present for several days and the animal was near collapse, the serum had no perceptible beneficial effect. This was, of course, to be expected in view of the incidence of various pathological phases of this disease—nephritis, hepatitis, and other toxic symptoms in succession. In man the clinical manifestations are more gradual and distinct than in the

guinea pig,³ yet the yellow fever patient whose temperature is subnormal, and who has reached the stage of hemorrhages from the gums, nose, stomach, and intestines, and of uremia and cholemia, would seem to have little or no chance of deriving benefit from the use of a specific immune serum. This latter assumption would probably hold irrespective of the relation which *Leptospira icteroides* proves to have to the etiology of yellow fever.

³ Noguchi, H., *J. Exp. Med.*, 1919, xxix, 547.