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ETIOLOGY OF YELLOW FEVER.

XIV. DURATION OF THE PROTECTIVE EFFECT OF ANTI-ICTEROIDES IMMUNE SERUM AFTER SUBCUTANEOUS INOCULATION INTO ANIMALS.

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As has already been reported,¹ the anti-*icteroides* immune serum is capable of protecting susceptible animals against infection with *Leptospira icteroides* when administered simultaneously or during the early period of the disease. Lyster, Pareja, Bailey, Vaughn, Vasconcelos, Casarus, Iglesias, Loyo, Le Blanc, Lynn and his associates, Hernandez, Lara, Villamil, Kligler, and Gann² have found that the mortality among human cases of yellow fever treated with the immune serum on or before the 3rd day of disease is much lower than that among untreated cases. Of 71 cases treated within the first 3 days of disease in Guatemala, Salvador, Honduras, Vera Cruz Merida, and northern Peru only 5 died (7 per cent mortality); the results of serum treatment were less favorable in the Tuxpam (Mexico) epidemic, where the mortality among the treated was 25 per cent (9 deaths among 36 cases), but here the mortality among untreated cases was correspondingly higher, being 68.6 per cent (59 deaths

¹ Noguchi, H., Serum treatment of animals infected with *Leptospira icteroides* *J. Exp. Med.*, 1920, xxxi, 159; Chemotherapy versus serotherapy in experimental infection with *Leptospira icteroides*, 1920, xxxii, 381. Noguchi, H., and Kligler, I. J., Immunological studies with a strain of leptospira isolated from a case of yellow fever in Merida, Yucatan, *J. Exp. Med.*, 1920, xxxii, 627; Immunology of the Peruvian strains of *Leptospira icteroides*, 1921, xxxiii, 253.

² Noguchi, H., Prophylaxis and serum therapy of yellow fever, *J. Am. Med. Assn.*, 1921, lxxvii, 181. See also the report of The Rockefeller Foundation for the year 1921.

among 86 cases). Of the total 187 cases treated to date, 107 received the serum on or before the 3rd day, and of these, only 14 died (mortality 13 per cent); on the other hand, there have been 41 deaths (51 per cent mortality) among 80 cases treated on or after the 4th day, and 225 deaths (56.6 per cent mortality) among 397 untreated cases occurring in the same localities during the same epidemic periods.

The serum has apparently undoubted therapeutic value in human cases of yellow fever as well as in cases of experimental infection with *Leptospira icteroides* in animals. Vaccination by means of killed cultures of *Leptospira icteroides* (injected in two subcutaneous injections of 2 cc. each, 4 to 6 days apart) has been shown to confer complete protection within 10 to 15 days of the last inoculation.^{2,3} During the period required for development of active immunity, however, anti-*icteroides* serum might be utilized for the immediate protection of non-immune individuals who find themselves in an epidemic or endemic focus of yellow fever, or it might be substituted for vaccination in the case of persons who intend only to pass through an infected district. It is well known that an immune serum or antitoxin, when introduced into the system of a non-immune individual, will protect against infection for a period which varies from a few to many days according to the number of units of immune bodies initially introduced and the rate of elimination of such bodies from the inoculated individual. The passive immunity thus conferred is necessarily of short duration, yet the employment of anti-*icteroides* serum for the temporary protection of non-immune individuals might be of considerable practical value.

In the experiments to be reported here guinea pigs were used to determine the duration of the protective effect of an injection of anti-*icteroides* immune serum.

EXPERIMENTAL.

Six different doses of immune serum (0.00001, 0.0001, 0.001, 0.01, 0.1, and 1 cc.) were subcutaneously inoculated into six sets (two each) of guinea pigs of about 500 gm. body weight. The tests for the per-

³ Noguchi, H., and Pareja, W., Prophylactic inoculation against yellow fever, *J. Am. Med. Assn.*, 1921, lxxvi, 96.

sistence of passive immunity were made 1, 2, 3, 4, 5, 7, 10, and 15 days after the injection of the immune serum. In order to infect all the guinea pigs, including six normal control animals, with the same material at the same time, the injections of the immune serum were begun 15 days before the time selected for the test inoculation of a virulent strain of *Leptospira icteroides*. The infective material used was an emulsion of the liver and kidneys of a guinea pig fatally infected with a strain of *Leptospira icteroides* isolated in Morropon by Noguchi and Kligler,⁴ which killed the control guinea pigs within 9 to 10 days in quantities of 0.001, 0.01, and 0.1 cc. 0.1 cc. of the emulsion, representing about 100 minimum lethal doses, was used in this experiment and was given subcutaneously. The results are recorded in Table I.

Analysis of the table shows that the minimum quantity of anti-*icteroides* serum required for protection, as revealed by the results obtained when the serum was given an hour previous to the inoculation of the infecting material, lay between 0.00001 and 0.0001 cc. Judged from the survival of three guinea pigs which received serum 24, 48, and 72 hours previously the protective titer of the serum must be close to 0.0001 cc. There was no diminution in the protective effect within 48 hours, but after 3, 4, and 5 days 0.0001 cc. no longer gave complete protection, while 0.001 cc. protected in every instance. 7 days after the time of injection of the serum 0.001 cc. failed to protect, but 0.01 cc. was still effective. After the lapse of 10 days 0.1 cc. was required to prevent infection, and after 15 days only those animals which had received 1 cc. of the immune serum withstood infection. The rate of elimination of the immune substance in the body of the guinea pig after the subcutaneous introduction of the anti-*icteroides* serum does not proceed uniformly in the successive days following injection, but follows a characteristic course which may be roughly estimated in the manner shown in Text-fig. 1, the unit being 0.0001 cc., which neutralized at least 100 minimum lethal doses in the present series.

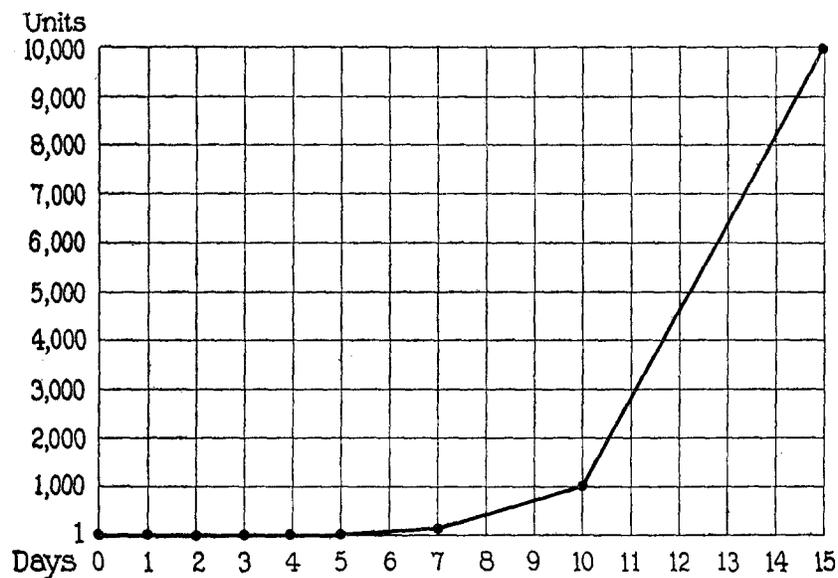
The rate of disappearance of the immune substance is very slow at first, but becomes rapid after about 10 days. This phenomenon is

⁴Noguchi, H., and Kligler, I. J., Experimental studies on yellow fever in northern Peru, *J. Exp. Med.*, 1921, xxxiii, 239.

TABLE I.
Results of Inoculation of 100 Minimum Lethal Doses of Leptospira icteroides into Guinea Pigs Previously Injected with Anti-icteroides Immune Serum.

Amount of serum injected.	Intervals between the injection of the immune serum and the inoculation of <i>Leptospira icteroides</i> .									
	1 hr.	24 hrs.	48 hrs.	3 days.	4 days.	5 days.	7 days.	10 days.	15 days.	
0.00001	+ (Killed for virus.)	-	-	-	Died in 9 days.	Died in 10 days.	Died in 10 days.	Died in 11 days.	Died in 11 days.	Died in 10 days.
	+ (Recovered.)	+ (Recovered.)	Died in 9 days.	Died in 9 days.	Died in 10 days.	Died in 10 days.	Died in 13 days.	Died in 10 days.	Died in 10 days.	Died in 10 days.
0.0001	-	-	-	-	+ (Killed.)	Died in 9 days.	Died in 11 days.	Died in 10 days.	Died in 10 days.	Died in 10 days.
	-	-	-	+ (Recovered.)	+ (Recovered.)	Died in 11 days.	+ (Recovered.)	Died in 13 days.	Died in 11 days.	Died in 11 days.
0.001	-	-	-	-	-	-	Died in 14 days.	Died in 10 days.	Died in 11 days.	Died in 11 days.
	-	-	-	-	-	-	+ (Recovered.)	Died in 16 days.	Died in 12 days.	Died in 12 days.
0.01	-	-	-	-	-	-	-	+ (Recovered.)	-	-
	-	-	-	-	-	-	-	(Recovered.)	Died in 12 days.	Died in 11 days.

not characteristic of anti-*icteroides* serum alone, because various immune serums appear to undergo a similar reduction in strength when introduced into different species of animals. Knorr, for example, found that tetanus antitoxin (horse serum), given to a foreign species (guinea pigs or rabbits), is reduced rapidly and that only about $\frac{1}{100}$



TEXT-FIG. 1. Curve showing the increase in amount of anti-*icteroides* serum (in units) necessary for protection according to the length of time elapsing after administration.

of the quantity originally present in the blood can be demonstrated after 12 to 14 days.⁵ Tizzoni⁶ obtained similar results. Nocard⁷ estimated that the longest period that the tetanus antitoxin remains

⁵ Knorr, A., Das Tetanusgift und seine Beziehungen zum thierischen Organismus. Eine experimentelle Studie über Krankheit und Heilung, *Münch. med. Woch.*, 1898, xlv, 362; Die Entstehung des Tetanusantitoxins im Thierkörper und seine Beziehung zum Tetanusgift, *Fortschr. Med.*, 1897, xv, 657.

⁶ Tizzoni, G., Sull' efficacia dell' antitossina nel trattamento preventivo contro il tetano dopo avvenuto l'infezione, *Gazz. osp.*, 1897, xviii, 1215.

⁷ Nocard, E., Sur la sérothérapie du tétanos; essais de traitement preventif, *Bull. Acad. med.*, 1895, xxxiv, 407.

in the body is about 4 to 6 weeks. Pfeiffer and Friedberger⁸ found that when an anticholera serum derived from the goat is injected into the rabbit it gives rise to an anti-immune substance by which it is neutralized in a comparatively short time. Ransom and Kitashima⁹ showed that homologous tetanus antitoxin, when injected into a normal horse, may remain as long as 80 days; that is, the duration of the passive immunity is almost as long as that of active immunity. On the other hand, Jørgensen and Madsen found that not all homologous immune serums remain for a long period in the body of the injected animal, but that the length of time varies with different animal species.¹⁰ Kraus and his coworkers failed to demonstrate any evidence for the formation of an anti-immune substance in the blood or organs of animals which had received an injection of a heterologous diphtheria antitoxin.¹¹ Perhaps the sudden disappearance of the immune substance after about 10 days in the present series of experiments may be intimately connected with the precipitin formation for the heterologous anti-*icteroides* horse serum. Moreover, the titer of the immune serum is certain to suffer reduction when kept at a temperature of 39°C., even *in vitro*, and may be expected to undergo similar reduction in the blood of a foreign species.

SUMMARY.

Analysis of the records of instances in which non-immune persons contracted yellow fever notwithstanding vaccination shows that the onset of disease occurs soon after vaccination, the longest period being 13 days. Since the average incubation period in yellow fever is 6 days, it seems that infection must have taken place in some instances during the period while protection was developing. These instances

⁸ Pfeiffer, R., and Friedberger, E., Ueber den Verbleib der bacteriolytischen Immunkörper im tierischen Organismus nach der passiven Immunisierung, *Centr. Bakt., 1te Abt., Orig.*, 1904, xxxvii, 131.

⁹ Ransom, F., The conditions which influence the duration of passive immunity, *J. Path. and Bact.*, 1900, vi, 180.

¹⁰ Jørgensen, A., and Madsen, T., The fate of typhoid and cholera agglutinins during active and passive immunisation, *Festskrift ved Indvielsen af Statens Serum-Institut*, 1902, Copenhagen, Paper 6.

¹¹ Kraus, R., and Joachim, J., Zur Frage der passiven Immunisierung, *Wien. klin. Woch.*, 1903, xvi, 1389.

led to a study of the possibility of immediate protection by means of the anti-*icteroides* serum. It had already been shown that the immune serum protects at once against experimental *Leptospira icteroides* infection, but it remained to determine how long the protection would last.

Guinea pigs were given different quantities of the immune serum and subsequently injected, at various intervals, with a virulent strain of *Leptospira icteroides*. Complete protection enduring 5 days was obtained with as minute a quantity of serum as 0.002 cc. per 1,000 gm. of body weight. After 5 days, however, the immune substance rapidly diminished, and to keep the animal protected for as long as 10 days it was necessary to give 100 times as much, or 0.2 cc. For a man weighing 80 kilos, 0.16 cc. (0.002×80) would theoretically be sufficient to protect for at least 5 days, 1.6 cc. for 7 days, and 16 cc. for 10 days. This temporary protection may be a valuable antecedent to that furnished by vaccination, since the final effect of the latter cannot be expected until at least 9 to 10 days have passed.