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ETIOLOGY OF YELLOW FEVER.

VII. Demonstration of Leptospira icteroides in the Blood, Tissues, and Urine of Yellow Fever Patients and of Animals Experimentally Infected with the Organism.

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The number of *Leptospira icteroides* found in the blood of yellow fever patients was so small that a prolonged examination of blood specimens was necessary to discover one organism, even when positive transmission had been obtained by injection of the blood into guinea pigs. In most cases the organism could not be detected in the blood, perhaps owing to lack of time. To devote much time to microscopic examination during the period while the work with yellow fever patients was being carried on was impossible and also inadvisable, since it was a matter which could be taken up at a later date when more time was available. Hence, what will be reported in this paper is not final but is the preliminary account of what was accomplished under the circumstances. The demonstration of the organism in the blood and various organs of animals experimentally infected with *Leptospira icteroides*, on the other hand, was much more satisfactorily accomplished. The infection could be produced at the desired time and the material obtained in sufficient quantity at any stage of the disease.

Examination of Material from Yellow Fever Cases.

As already stated[1] a minute organism belonging to the genus *Leptospira* has been found both in the blood and in the tissues of various organs, particularly the liver and kidneys, of guinea pigs

[1] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 565.

which contracted an experimental infection after being inoculated with the blood of yellow fever patients taken during the early stages of the disease. In three instances (Cases 1, 4, and 6) the organism was found in the cultures made directly from the blood of yellow fever patients. A special effort was made, therefore, whenever a case of yellow fever was admitted to the hospital or an autopsy was performed, to find the same organism in the blood and tissues, and if possible also in the urine.

The dark-field microscope was used for examination of the fresh material. Films and sections were stained by Giemsa's or Wright's stain, by Levaditi's or Noguchi's silver impregnation methods, as occasion demanded. Fontana's silver impregnation method was applied several times without satisfactory results. Blood films were usually fixed with methyl alcohol,[2] while the impression films of various tissues from autopsies were first fixed over osmic acid vapor for 1 minute and then in absolute alcohol for 30 minutes. They were stained with Wright's stain for 30 minutes and then with Giemsa's stain over night. Six to twelve were prepared from each specimen of blood or tissue for examination.

Of twenty-seven cases of yellow fever in only three (Cases 3, 4, and 14) was the leptospira demonstrated in the blood under the dark-field microscope, and in each instance the number of the organisms found was so small that once they had passed out of the field they were not easily encountered again. The blood of two of these three patients (Cases 3 and 4) also yielded positive transmission into guinea pigs.[1] The organism was found in a few of the stained film preparations, but these will have to be repeatedly examined. Some specimens of yellow fever blood, however, were infectious for guinea pigs even when in the fresh state no leptospira could be demonstrated under the dark-field microscope (Cases 1, 2, 5, and 6). That there must have been a very small number of the organisms in such specimens is shown by the fact that the blood and organs of the infected animals contained the organisms. A negative microscopic examination of the blood indicates either absence or scarcity of the organism. Nor does the failure of the blood to reproduce a fatal experimental

[2] For the purifying of the methyl alcohol for this purpose I am indebted to Dr. Herman Edward Redenbaugh of the Commission.

infection in guinea pigs prove the absence of the organism in the specimen, since variations in its pathogenicity for guinea pigs are considerable (Case 14).

Examination of various organs under the dark-field microscope has yielded so far only one positive finding, that in the liver of Patient J. Co. (Case 4), who died on the 4th day of the disease. The liver was excised from the body (a partial autopsy) a few hours after death while the body was still quite warm. The kidney failed to show any leptospira. In the stained specimens of the blood, liver, and kidney a small number of leptospiras was demonstrated. Both the blood and the liver emulsion of this patient yielded a positive transmission of the disease to guinea pigs.[1]

A careful search for the organism was carried out with the films made from the liver, kidney, lungs, adrenals, mesentery, and inguinal glands from ten more cases, with so far a positive finding in the liver of one case (Case 5). Some of these slides were poorly stained and will have to be repeatedly examined when more time is available. In a later paper will be recorded the results of the examination of sections of the organs from patients who died of yellow fever.

Specimens of urine from twenty-one cases of yellow fever were examined under the dark-field microscope, but no leptospira was encountered. The examinations were made during the height of the illness, which is usually the 5th or 6th day of the disease, as well as during convalescence, towards the end of the 2nd week. This part of the investigation was much handicapped by the lack of a powerful centrifuge to concentrate the urine, and it will have to be repeated under more favorable conditions. As might be expected, *Treponema minutum*[3] and *Treponema calligyrum*[4] were occasionally seen in the samples of urine. A doubtful result was obtained in a guinea pig inoculated with 10 cc. of the urine from a convalescent (Case 51) on the 15th day of the disease, although no leptospira was found in the specimen.

[3] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 667.
[4] Noguchi, H., *J. Exp. Med.*, 1913, xvii, 89.

Examination of Material from Experimentally Infected Animals.

Experiments were undertaken to determine the approximate time when the blood, liver, and kidney of guinea pigs infected with the yellow fever virus first contain enough organisms to produce the infection when transferred to a normal guinea pig, and when they cease to be infective. These points are more of academic interest, as they do not affect the present sanitary measures with regard to isolation of the patients before, during, and after the actual sickness.

When a minute quantity of the virus is introduced into the subcutaneous tissue of the guinea pig no local reaction follows. The blood becomes infective as early as 48 hours after the inoculation in some instances, but more constantly so after 72 hours. The liver and kidney become infective simultaneously with the blood. The amount of blood effective for infection is comparatively large, at least 1 cc. being required during the earlier period. The demonstration of the organism in the blood is almost hopeless and has not been successful until the 5th day after inoculation, when occasional specimens have been found after a long search. The organisms are more readily found in the emulsions of the liver or kidney, sometimes more numerously in one and sometimes in the other. After the onset of the disease the number of organisms gradually increases for a time, both in the blood and in the liver and kidney. Then the relation of the organism to the blood on the one hand and to the liver and kidney on the other seems to show a certain difference. The organisms continue to increase in the organs somewhat longer than in the blood. In fact, the number of organisms in the blood becomes fewer as the disease progresses, and only a few can be detected when the jaundice and other symptoms have fully developed; that is, a day or two before death. This has been the rule with the majority of the guinea pigs in the present experiments, but there were exceptions in which the organisms were quite numerous until death. Although they increase in the liver and kidney in greater number and for a longer period, they have been found in most instances finally to disappear also from these organs. The disappearance is sudden and usually occurs about 24 hours before death, although there are instances in which they can be found in large numbers at death, sometimes

more abundantly in one or the other organ. Although it is true that when the blood contains the leptospira at the time of death the liver and kidney also contain it, yet the reverse is not always the case, the organism being found in the organs but not in the blood. An instance has not been met in which the leptospira was present in the blood but not in the liver or kidney or both.

The mechanism of the disappearance of the leptospira in so many typical experimental guinea pigs during the later stage of the infection is difficult to explain, but it may be partly due to the formation of certain still undetermined metabolic products brought about by the disorganization of the liver or kidney or some other organs. The leptospira is highly sensitive to the dissolving action of bile salts *in vitro*, and it is not inconceivable that a predecessor or a derivative of taurocholic, glycocholic, or cholic radicals in a certain stage may exert a powerful destructive action upon it.

With regard to the question of the infectivity of the blood and organs in the later stage of the infection, the statement may be made that in all instances in which the organism was seen, whether in the blood or in the organs, the material was always infective. On the other hand, in a considerable number of instances in which the dark-field search for the organism failed, no infection could be induced in normal guinea pigs by inoculation of the material. It may be supposed that the organisms had completely disappeared from the body in these cases. However, as was to have been expected, there were many instances in which, notwithstanding an unsuccessful microscopic search for the organism, inoculation of the liver or kidney, or both, but not the blood, gave rise to a typical infection in further passage, with the reappearance of the leptospira in the new host. In one instance the leptospira was microscopically detected on the third passage. It may be concluded, then, that in experimental infection in the guinea pig the specific organism survives longer in some of the infected hosts than is assumed to be the case in yellow fever in man.

SUMMARY.

Examinations of fresh blood from yellow fever patients by means of the dark-field microscope, made in more than twenty-seven cases,

revealed in three cases the presence of *Leptospira icteroides*. In no instance was a large number of organisms found, a long search being required before one was encountered. The injection of the blood into guinea pigs from two of the three positive cases induced in the animals a fatal infection, while the blood from the third positive case failed to infect the guinea pigs fatally. Careful but by no means exhaustive dark-field searches for the leptospira with fresh specimens of blood from the remaining cases of yellow fever ended without positive findings, although four of the specimens, when injected into guinea pigs, caused a fatal leptospira infection.

Stained blood film preparations from the corresponding cases were also examined, but the percentage showing the leptospira in the blood was no greater than that found by examination in the fresh state with the dark-field microscope. In fact, owing to the defective stains that were available at the time of the investigation a great many slides did not take the proper coloration with Giemsa's or Wright's stain and could not be relied upon.

Regarding the presence of *Leptospira icteroides* in various organs both dark-field and stained films were examined. In only one instance so far a few organisms were detected in the emulsion of liver taken shortly after death from a case dying on the 4th day of yellow fever. This part of the work will be reported later upon completion.

Examinations of the urine from different cases of yellow fever were made both by dark-field microscope and by inoculation into guinea pigs. The results were totally negative in thirteen cases, including many convalescents, but in one case one of the guinea pigs inoculated with 10 cc. of the urine came down on the 15th day with suggestive symptoms (suspicion of jaundice, and some hemorrhagic and parenchymatous lesions of the lungs and kidneys). This specimen showed no leptospira by dark-field examination.

In experimental infection of guinea pigs with *Leptospira icteroides* the blood became infective in many instances 48 hours after inoculation, and was always infective after 72 hours. The liver and kidney become infective simultaneously with the blood. Detection of the organism by means of the dark-field microscope has seldom been accomplished before the 5th day. The organisms are most abundant on the 6th to the 7th day, but become fewer or completely

disappear before death. In the meanwhile the number of organisms increases in the liver and kidney, from which they disappear as the jaundice and other symptoms become aggravated. When death occurs these organs seem to have lost most of the leptospira, and positive transfer by means of them is less certain. At the later stage of the disease the blood is often free from the organisms and ceases to be infective. Positive transmission with blood obtained from moribund animals is not impossible, however, even when no leptospira can be detected under the dark-field microscope.