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THE SURVIVAL OF LEPTOSPIRA (SPIROCHÆTA) ICTERO-HÆMORRHAGIÆ IN NATURE; OBSERVATIONS CONCERNING MICROCHEMICAL REACTIONS AND INTERMEDIARY HOSTS.

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A solution of the question of the survival of *Leptospira icterohæmorrhagiæ* in nature may be brought about (1) by following up directly the actual conditions to which the spirochetes cast off by the hosts or artificially mixed with urine or feces will have to submit, or (2) by mixing the spirochetes with each in turn of the various bacteria commonly encountered in feces, sewage, or soil, and then determining the results of their simultaneous existence in the same media. As I shall show, the spirochete of infectious jaundice is a very delicate organism and is rapidly overwhelmed by most of the bacteria from intestinal contents, sewage, or soil.

Urine in Relation to Leptospira icterohæmorrhagiæ.

The effect of urine upon the viability of *Leptospira icterohæmorrhagiæ* is of practical importance, since it has been found by previous investigators[1, 2, 3] that the urine of about 77 per cent of the patients recovering from infectious jaundice still contains the spirochete after a period of 2 to 5 weeks. They made the interesting observation that two-thirds of the positive urines, some containing numerous spirochetes, failed to produce the infection in guinea pigs. Since the urines came from cases of 15 days' standing or longer, the fact may be ex-

[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1917, xxvi, 341.
[2] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 38.
[3] Cappellani, S., and Frugoni, C., *Sperimentale*, 1917, lxxi, 335.

plained by an attenuation in virulence of the organism during the course of the disease. I have seen one case in which the urine was still infective for guinea pigs 1 month after the onset of the disease.

In one series of experiments a sample of urine freshly collected from a healthy individual, who had no history of ever having had jaundice, was tested for its action upon the spirochetes. 10 cc. amounts of

TABLE I.

Leptospira icterohæmorrhagiæ introduced into.	Changes in appearance and reaction.	0.2 cc. of a well growing culture of American strain No. 1 inoculated into tubes containing.				
		Fluid indicated 6 cc.		Fluid indicated 3.5 cc. + rabbit serum 1.5 cc. + citrate plasma 1 cc.		
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	3 days.
Normal urine	Clear, strongly acid.	−	−	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.1 cc.	Clear, neutral.	<+	−	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0.2 cc.	Precipitate +, slightly alkaline.	<+	−	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0 4 cc.	Precipitate ++, moderately alkaline.	<+	−	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.8 cc.	Precipitate +++, markedly alkaline.	−	−	−	−	−

the urine, which was strongly acid and had a titer such that 10 cc. of it required 7 cc. of 0.1 N sodium hydroxide to become moderately alkaline, were measured into a number of test-tubes, to each of which was added normal sodium hydroxide solution, the quantities added varying in each case in order to obtain a series of reactions from the original acidity of the specimen to a markedly alkaline reaction.

Cultures were set up in two parallel series, using in each series the original and partially neutralized portions of the urine, but adding to one series suitable amounts of rabbit serum and citrate plasma. Table I summarizes the results.

As the table shows, the jaundice spirochetes survived at least 24 hours in the portion of urine to which a quantity of from 0.1 to 0.4 cc. of normal sodium hydroxide solution had been added, but no trace of them could be found in the original urine or in that receiving 0.8 cc. of the alkali. In plain Ringer's solution alone the organism lived 24 hours under similar conditions. After 48 hours there were no spirochetes in any of the tubes of the first series.

The results obtained with the urine containing rabbit serum and citrate plasma were different from those of the other series. There was a good growth in all the tubes containing the unalkalized urine, and also in those to which had been added from 0.1 to 0.4 cc. of normal sodium hydroxide solution. The growth was better and lasted longer in the tubes in which the urine showed a neutral or slightly alkaline reaction than in the unmodified or more strongly alkalized urines. There was no growth in the tube to which 0.8 cc. of the normal sodium hydroxide had been added. While there was unmistakable growth in the urine media with rabbit serum and citrate plasma, the organisms were viable for only 1 week at the longest. The presence of the urine apparently reduces very much the nutrient value of the rabbit serum and citrate plasma, as is shown by the fact that the use of Ringer's solution instead of urine enables the spirochetes to multiply progressively for at least 3 weeks. Not only is the urine devoid of cultural value for the organism, but its presence in an otherwise suitable medium renders the latter less suitable for the growth of the organism.

Feces in Relation to Leptospira icterohæmorrhagiæ.

The escape in feces of living *Leptospira icterohæmorrhagiæ* from experimentally infected guinea pigs seems to be rather frequent. Ido and his coworkers,[1] for example, succeeded in producing typical spirochetosis in seven out of eleven animals tested with a corresponding number of specimens of feces; yet in spite of this high percentage of

TABLE II.

Specimens of feces from which media were prepared.	1:10 dilution.		1:10 dilution, autoclaved.		1:100 dilution, filtrate.	
	No blood.	Blood and serum added.	No blood.	Blood and serum added.	No blood.	Blood and serum added.
Normal feces No. 1	24 hrs.: A few distorted spirochetes among innumerable bacteria. No trace of the spirochetes after 48 hrs.	Same as preceding.	A few motile spirochetes after 24 hrs. but none after 48 hrs. No difference between normal and jaundice feces.	Moderate multiplication of spirochetes in 4 days, which gradually increased. Four daily inoculations (intraperitoneal) all positive.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily inoculations all positive.
" " " 2	Four daily inoculations (scarified skin) in guinea pigs all negative.		Four daily inoculations (intraperitoneal) in guinea pigs all negative.	Moderate multiplication of spirochetes in 4 days, which gradually increased. No animal inoculations.	Spirochetes survived 4 days, after which they disappeared. No animal inoculations.	A good growth in 4 days which progressed well for 10 days. No animal inoculations.
Jaundice feces No. 1				Moderate multiplication of spirochetes in 4 days, which gradually increased.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily animal

Jaundice feces No. 2					Four daily inoculations. All positive. Moderate multiplication of spirochetes in 4 days, which gradually increased. No animal inoculations.	One 1st day inoculation positive. Spirochetes survived 4 days, after which they disappeared. No animal inoculations.	inoculations. All positive. A good growth in 4 days which progressed well for 10 days. No animal inoculations.

positive results, it was by no means easy to demonstrate the presence of the spirochetes in feces with the dark-field microscope, the organism being found only once in the stools from 60 guinea pigs having spirochetosis. The specimens of feces which were infective usually contained erythrocytes. In human cases one specimen of feces out of seven was found to be infective.

There is apparently a possibility, then, that the spirochetes are excreted from patients in the feces. To determine the viability of the spirochetes under these conditions, a series of experiments was performed in which they were added in large quantities to feces, both with and without a simultaneous addition of blood ingredients. Two specimens of feces from normal individuals were used, and two from cases of jaundice in children, with the characteristic clay color. Each specimen was used in three ways: (1) as a moderately thick emulsion (1:10) in Ringer's solution, without sterilization; (2) the same sterilized by autoclave; (3) a Berkefeld V filtrate of a dilute fecal emulsion (1:100). Two sets of tubes were set up, each containing 5 cc. of one of the variously prepared suspensions. To each of the tubes of one set were added a few drops of defibrinated blood and 1 cc. of serum from a normal rabbit, and to all the tubes were added 2 cc. of a richly growing culture of either the American or the European strain of the organism. Both sets of tubes were then placed at a temperature of 26°C.

The fate of the spirochetes under these conditions was followed daily by direct microscopic examination and indirectly by inoculation tests on guinea pigs. Table II summarizes the results obtained with the European strain. The results with the American strain were practically identical and are consequently not recorded here.

As is apparent from the table, the spirochete cannot survive in a fecal emulsion, even when there are present sufficient nutrient elements, longer than 24 hours at a temperature of 26°C. That this fact is due to the simultaneous presence of various bacteria, which rapidly overgrow the delicate spirochetes and deprive them of the necessary nutrient substances, is inferred from the much longer survival of the spirochetes in the tubes containing the sterilized emulsion, particularly in those to which were added the blood and serum. In the latter tubes, in fact, there was a temporary multiplication of the

organisms lasting several days. In the tubes containing a dilute, sterile, fecal filtrate, the spirochetes survived at least 4 days, and the addition of blood and serum caused the filtrate to become a suitable culture medium, if not equally as good as Ringer's or saline solution. Inoculations made with the mixtures of the non-sterilized fecal emulsions, with and without the blood and serum and the spirochetes, applied to the scarified skin of guinea pigs, were all negative. Where there was an actual multiplication or survival of the organism, as in the case of sterile suspensions or filtrates, with or without the addition of blood, the animal inoculations were positive.

Judging from the foregoing experiments, it seems highly improbable that, under natural conditions, the causative agent of icterohemorrhagic spirochetosis survives for any length of time after it has left the human body in the feces. It is probably rapidly destroyed by the common bacterial flora of the intestinal tract.

Polluted Water and Soil in Relation to Leptospira icterohæmorrhagiæ.

Samples of water were collected from the East River (a tidal river), from sewage, and from a stagnant cesspool in New York City. It is needless to say that such water is highly contaminated with various bacteria. In one series of experiments the water was used as it was, in another it was autoclaved in order to destroy contaminating bacteria, and in another it was filtered. An emulsion of freshly excreted horse stool was used in one series. The experimental data are given in Table III.

The results show that the spirochetes are not capable of multiplying or even of surviving for any length of time in these contaminated waters. They invariably disappeared in 48 hours. Even when the contaminating bacteria were removed by autoclaving or filtration and rabbit serum was added, only indifferent media resulted, and without the addition of an adequate amount of a suitable nutrient medium (rabbit serum in this experiment) no culture could be obtained.

The question of how long a rich culture of the spirochetes will remain viable when mixed with distilled water and left unprotected from dust in a room was next determined. A Flanders strain, having grown luxuriantly in rabbit serum, Ringer's solution, and agar mix-

TABLE III.

Leptospira icterohæmorrhagiæ introduced into.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.
Ringer's solution 4.5 cc. + rabbit serum 1.5 cc. (control)..	−	+++	Many wks.
East River water 6 cc.	+++	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " " autoclaved, 6 cc.	−	−	
" " " " 4.5 cc. + rabbit serum 1.5 cc.	−	+	2 wks.
Sewer water 6 cc.	+++	−	
" " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " autoclaved, 6 cc.	−	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	−	+++	Many wks.
Stagnant water 6 cc.	+++	−	
" " 4.5 cc. + rabbit serum 1.5 cc	+++	−	
" " autoclaved, 6 cc.	−	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	−	+++	Many wks.
Horse stool emulsion 6 cc.	+++	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " " autoclaved, 6 cc.	−	−	
" " " " 4.5 cc. + rabbit serum 1.5 cc.	−	+(?)	Accidentally contaminated.
Sewer filtrate 6 cc.	−	−	
" " 4.5 cc. + rabbit serum 1.5 cc.	−	++	More than 3 wks.

ture for 22 days, was placed in distilled water (ten times the volume of the culture) and then allowed to stand in the laboratory without being covered. The distilled water was not sterile, but contained a few large motile bacilli. The results were as follows:

24 hrs: Spirochetes +++; active and long; numerous motile bacilli; fluid slightly opalescent.
48 hrs.: Spirochetes +++; active; more bacilli.
3 days: " ++; " " "
4 " " +; " probably more bacilli.
5 " " +; many immobile; " "
6 " " <+; nearly all dead.
7 " " −.

The spirochetes remained active and numerous for 48 hours, but all of them gradually disappeared within a week. A drinking water, therefore, richly contaminated with spirochetes, will not be infectious longer than a week.

Samples of soil were collected from several localities in and about New York City for use in an experiment performed to ascertain how long soil will harbor spirochetes under experimental conditions. The samples were rich in organic matter and some came directly from fertilized ground. They were all neutral in reaction. One specimen of soil was obtained from a deeper stratum than the others and was yellowish gray in color. All were purposely contaminated with the spirochetes and determinations of their continued presence in it made daily. No spirochetes could be detected after 72 hours, while there was always an abundance of bacteria. The spirochetes seem to be rapidly overgrown by the contaminating bacteria.

Various Bacteria in Relation to Leptospira icterohæmorrhagiæ.

When the spirochetes are excreted from the infected host, either in the feces or in the urine, their immediate fate will depend upon the presence of various putrefactive bacteria which are always found in the soil in which the feces or urine is deposited. Today we know all of the more common varieties of bacteria that inhabit the intestinal tract or that may be found in unclean objects or soil. There are, of course, a great number of anaerobes as well as aerobes, but since the spirochete in question is an obligatory aerobe,[4] the study of the relation of the bacteria to it becomes much simpler. We have, therefore, to direct our attention only to the part played by aerobic bacteria under natural conditions.

There are many ways of conducting such a study, but I have chosen an indirect one; namely, that of observing the effect of the simultaneous presence of the spirochete in question and each in turn of those bacteria which are likely to coexist with it at the moment when the infected feces, urine, or dead rodent becomes subject to the decomposing forces of the organic world.

A number of culture tubes containing media suitable for the growth

[4] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 593.

of spirochetes was prepared, and all were inoculated with the organism. The tubes were then inoculated with various bacteria and placed in a

TABLE IV.

Bacteria.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.	Remarks as to hemolysis in media.
Control without bacteria	−	+++	Many wks.	−
B. fæcalis alkaligenes	+	+++	12 days.	−
B. aerogenes	++	−	48 hrs.	+
B. cloacæ	++	−	24 "	<+
B. coli	++	−	24 "	<+
B. dysenteriæ Shiga	+	<+	48 "	−
B. " Flexner-Harris	<+	+	48 "	−
B. typhosus	<+	<+	48 "	−
B. paratyphosus A	++	−	24 "	−
B. " B	++	−	24 "	−
B. prodigiosus	++	−	24 "	+
B. proteus vulgaris	++	−	24 "	+
B. pyocyaneus	++	−	24 "	+
B. suipestifer	++	−	24 "	−
B. suicidus	++	−	24 "	−
B. subtilis	++	−	24 "	<+
B. mesentericus	+	−	24 "	<+
B. xerosis	+	−	24 "	−
B. sp.? large, motile, chromogenous	+	++	4 days.	−
Streptococcus Pr	<+	++	5 "	−
" Brown F 17	++	−	24 hrs.	−
" " A 1	±	++	5 days.	−
" " C 2	+	++	8 "	−
" " W 18	++	<+	3 "	−
" " K 4	++	++	5 "	+
" " S 6	++	<+	6 "	+
" " H 6	+	−	24 hrs.	+
Pneumococcus Type I	++	<+	3 days.	−
" " II	++	−	24 hrs.	−
" " III	+	<+	3 days.	−
" " IV	+	−	24 hrs.	−
Streptococcus aureus	++	+	48 "	<+ slowly.
" *albus*	++	+	48 "	−

thermostat at the temperature of 26°C. The culture media consisted of 1.5 cc. of rabbit serum, 4.5 cc. of Ringer's solution, 1 cc. of citrate plasma, and 1 drop of defibrinated rabbit blood. Observations were

made of the growth, survival, or disappearance of the spirochetes, the growth of the bacteria, and the presence or absence of hemolysis in the cultures. The results obtained during a period of 2 weeks are recorded in Table IV.

It is apparent from the recorded observations that the more vigorous the growth of a bacterium, the less is the possibility that the spirochetes in the same medium will multiply. The longest period of survival of the spirochetes, except in the control tubes, was observed in the media simultaneously inoculated with *Bacillus fæcalis alkaligenes*. Certain strains of streptococci, notably the non-hemolytic types, seem not to have interfered for a certain period, after which, however, the spirochetes rapidly disappeared from the culture. In the presence of most of the intestinal bacteria, such as *Bacillus coli*, *Bacillus aerogenes*, *Bacillus cloacæ*, etc., the spirochetes were not only unable to multiply but were rapidly destroyed within 24 hours. It may be added that no growth of the spirochetes took place in ordinary bouillon, either with or without the simultaneous inoculation of the bacteria just enumerated. The bacteria grew vigorously in the bouillon.

Microchemical Reactions.

The resistance of various spiral organisms to the solvent action of bile, bile salts, saponin, and sodium oleate has been a subject of study for many years, and it was once thought to differentiate the protozoa from the bacteria. Although this view is no longer valid, because some bacteria have been found to act like protozoa and *vice versa*, the fact is of sufficient interest to make worth while a determination of the resistance of the present organism to these reagents (Table V).

The jaundice spirochetes appear to be highly sensitive to the destructive action of the bile[5] and bile salts when employed in concentrations of 1:30 or more, while saponin exhibited no injurious effect upon them, even when used in as high a concentration as 10 per cent. The action of sodium oleate was stronger than that of the bile or bile salts and produced a granular disintegration of the organism in a dilution of 1:10,000. Among the organisms which under-

[5] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 41.

TABLE V.

Reagent.	Results in different concentrations.									
	1:10			1:30		1:100			1:300	1:1,000
	After 5 min.	After 30 min.	After 5 hrs.	After 5 min.	After 5 hrs.	After 5 min.	After 30 min.	After 5 hrs.	After 5 hrs.	After 5 hrs.
Ox bile.	Still active.	None motile, nearly all shadow forms.			All shadow forms.			Some affected. Nearly all active.	No effect. All active.	
Rabbit bile.	Many inactive.	All shadow forms.			All shadow forms.			All shadow forms.	Some affected. Majority active.	
Sodium taurocholate.	All shadow forms.	Shadow forms.	Shadow forms less distinct.	All immobile. Better preserved in form.		Nearly all active.	All shadow forms.		Nearly all active.	No effect. All active.
Sodium glycocholate.	Nearly all shadow forms.		All shadow forms.		All shadow forms.			Some still active.	Nearly all active.	No effect. All active.
Sodium oleate.	All dead; distorted and granular.			All dead; distorted and granular.				Nearly all gone; few motile.	Nearly all gone; few motile.	Nearly all gone; few motile, but more active than those in the 1:300 dilution.
Saponin.			No effect. All active.		No effect. All active.			No effect. All active.	No effect. All active.	

went this disintegration, however, was a number of actively motile, apparently intact organisms.

The destructive action of the rabbit bile as well as of the bile salts and sodium oleate was considerably reduced by the addition of serum, as shown in Table VI.

Ido and his coworkers[1] observed that in spite of the difficulty of finding spirochetes in the bile when it was examined under the dark-field microscope, two out of three specimens of the bile of guinea pigs dying of experimental spirochætosis icterohæmorrhagica were capable of producing typical infection in the guinea pig. This

TABLE VI.

Leptospira icterohæmorrhagiæ introduced into.	10 per cent rabbit bile 1 cc. + culture 1 cc.	10 per cent sodium taurocholate 1 cc. + culture 1 cc.	10 per cent sodium oleate 1 cc. + culture 1 cc.	0 + culture 1 cc.
Rabbit serum 0.5 cc.	No apparent effect. All active.	Nearly all active.	Nearly all active.	All motile.
60 per cent rabbit serum 0.5 cc.	Many gone, some motile.	Many dead and distorted. A few motile.	Many active.	" "
20 per cent rabbit serum 0.5 cc.	Nearly all gone.	Nearly all gone.	" "	" "
6 per cent rabbit serum 0.5 cc.	All gone.	All gone.	Nearly all gone.	" "
2 per cent rabbit serum 0.5 cc.	" "	" "	All gone.	" "
Ringer's solution 0.5 cc.	" "	" "	" "	" "

may be ascribed to the fact that in these specimens of bile there was mixed a certain amount of the blood and also the serous exudate from the affected liver, which, by virtue of their well known inhibitory effect upon the solvent action of the bile salts, must have protected some spirochetes from destruction in the bile. Guinea pig bile was affected by the serum in the same way.

A parallel series of experiments with a specimen of ox bile obtained from an abattoir gave somewhat contradictory results. In this instance the addition of the rabbit or horse serum failed to check the destruction of the organism by this bile, which had a much stronger

solvent power than that of the rabbit or guinea pig. At all events, the amount of the serum necessary to nullify the destructive action of the bile is so large that the escape of the spirochetes in the bile seems less probable than would appear from the observations of the investigators just quoted. Perhaps the impairment of hepatic function through the spirochetal infection of the organ may lead to a decrease of the bile salts in such a specimen.

Leptospira icterohæmorrhagiæ and Intermediary Hosts.

It has been shown by previous investigators that the spirochetes may remain in the organs of certain rodents without producing serious illness, and that they may be excreted in the urine. From the experiments already described, it seems improbable that the spirochete can survive very long after leaving the infected hosts. The infection of man, therefore, must result from contact with the spirochete before its destruction under natural conditions; that is, the carrier rodents must be present in places frequented by man. But while this source of infection may explain many cases of infection, there are a few in which the infective agent cannot be traced in this way.

The question of insect carriers has been taken up by Reiter,[6] who obtained only negative results with certain biting flies, fleas, and bedbugs. In the present study opportunities were afforded the writer to ascertain whether or not the larvæ of certain varieties of flies or mosquitoes could become infected with spirochetes when fed on infected guinea pig liver or raised in a stagnant water tank into which an abundance of the culture had been put.

The larvæ of the common house-fly were allowed to feed for 2 days on infected material consisting of several pieces of the liver and kidney of a guinea pig killed in the last stage of experimental Weil's disease. They were then transferred to a clean receptacle and fed for 5 days on a non-infected mass of horse manure, and at the end of that time they were crushed into an emulsion and smeared over depilated areas of the skin of guinea pigs. The emulsion was also examined for spirochetes under the dark-field microscope. The examination revealed no spirochetes, and the guinea pig remained normal.

[6] Reiter, H., *Deutsch. med. Woch.*, 1916, xlii, 1282.

A similar experiment with the larvæ of bluebottle flies (*Calliphora vomitoria*) gave only negative results.

In another series of experiments, about 50 cc. of a rich culture of spirochetes (Japanese strain) were added to 150 cc. of stagnant water in which twenty-five mosquito larvæ had been living for some time. The water was neutral in reaction and was quite clear and transparent at the time when the culture was introduced. The larvæ swam about actively in the usual manner after the addition of the culture. A drop of the contaminated water examined under the dark-field microscope contained numerous active spirochetes. There were a few bacteria. After 24 hours at room temperature, the water became somewhat turbid. Most of the larvæ were still active, but the number of the spirochetes was diminished and that of the bacteria increased. At the end of 48 hours there was a scum of bacteria over the surface of the water and no spirochetes could be found. All but six of the largest larvæ had died. The water was full of bacteria and infusoria. It is possible that the death of the mosquito larvæ and of the spirochetes was the result of overcrowding by the bacteria and infusoria, increased suddenly by the addition of the culture media to the water. The surviving larvæ were kept in the same water for 5 days and then crushed into an emulsion to be used for an infection experiment on a guinea pig and also for examination under the dark-field microscope. The results were entirely negative.

Another series of experiments was performed with adult mosquitoes (*Culex pipiens*) by first allowing them to feed on an infected guinea pig, in the blood of which had been found spirochetes, and then, after 6 days, causing them to bite normal guinea pigs. No infection resulted from their bites.

Wood ticks (*Dermacentor andersoni*) failed in several experiments to transmit the infection from guinea pig to guinea pig. Leeches (*Hirudo medicinalis*) were allowed to suck blood from an infected guinea pig until their bodies were engorged. In the blood escaping from the wound inflicted by the leeches a few spirochetes could be found under the dark-field microscope. These "infected" leeches were kept at room temperature for 7 days and afterwards in a cool room at 15°C., being taken out at the end of intervals of 2, 3, 4, 6, and 8 months and made to suck normal guinea pigs, but so far no infection

has been produced. Some of the leeches died in the meantime, but those which still survived at the end of the 3 month interval were examined for the presence of spirochetes. The viscid, dark reddish, decomposed (?) blood showed no spirochetes under the dark-field microscope, nor did it cause infection when tested on guinea pigs. Some of the tissues were examined by the silver impregnation method, but with negative results. Apparently there is no multiplication of the spirochetes after their ingestion by leeches, and no infection can be induced by the bite of the latter after a period of 1 week.

SUMMARY AND CONCLUSIONS.

1. *Leptospira icterohæmorrhagiæ* is unable to grow in the urine, either with or without the addition of suitable culture ingredients, the acidity of the urine being detrimental to the growth. It survives less than 24 hours, unless the urine is neutralized or slightly alkalized, when the period of survival is somewhat longer. If suitable nutrient ingredients are added to the neutralized or slightly alkalized urine, the organism is able to grow for about 10 days, after which multiplication ceases.

2. Feces from normal or jaundiced persons destroy *Leptospira icterohæmorrhagiæ* within 24 hours when a rich culture is added and the mixture allowed to stand at 26°C. The addition of blood serum and corpuscles does not prevent the destruction of the organism. Autoclaved specimens and filtrates of unheated feces do not constitute a suitable medium in which to keep the organism alive for any length of time, but the addition of blood corpuscles and serum in adequate quantities renders them fairly satisfactory as media. Under natural conditions *Leptospira icterohæmorrhagiæ* cast off in the feces cannot survive more than 24 hours.

3. Polluted water, sewage, and soil will not serve to keep *Leptospira icterohæmorrhagiæ* alive for more than 3 days at the most. When deprived by filtration or autoclaving of their bacteria they become indifferent diluents and may be used to make up a culture medium when mixed with serum and citrate plasma of a suitable animal. Sterilized soil with a neutral reaction, when added to a culture, has an unfavorable effect upon the growth of the organism.

4. Most of the aerobic bacteria found in feces, sewage, soil, and tap water inhibit the growth of *Leptospira icterohæmorrhagiæ* when inoculated into the same medium. *Bacillus fæcalis alkaligenes* and many strains of non-hemolytic streptococci caused the least interference, although growth was never so vigorous or lasting in the media in which they were present as in the control media. Certain pathogenic bacteria (*Bacillus typhosus*, *Bacillus paratyphosus*, *Bacillus dysenteriæ*, pneumococcus) are antagonistic to the growth of the spirochete.

5. *Leptospira icterohæmorrhagiæ* is highly sensitive to the destructive action of bile, bile salts, and sodium oleate, but resists the action of saponin. In this last respect it differs from many so called spirochetes. The destructive action of these agents is counteracted by blood serum.

6. The larvæ and adults of the *Culex* mosquito, the larvæ of the house-fly and bluebottle fly, wood ticks (*Dermacentor andersoni*), and leeches failed to become carriers of the spirochetes when fed on infected guinea pigs or their organs; that is, they cannot play the part of an intermediary host of *Leptospira icterohæmorrhagiæ*.