Bull. Inst. Zool., Academia Sinica 4: 59-63 (1965)

THE INFLUENCIAL CONDITIONS OF KIDNEY TISSUE ON THE ISOLATION OF *LEPTOSPIRES*[1]

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Received for publication November 23, 1965

ABSTRACT

In the present experiment using 20 guinea pigs, the factors influencing the isolation of *Leptospires* from the kidneys of guinea pigs inoculated with *L. australis A* were studied by using Korthof's and Stuart's media to which pooled rabbit sera, free from growth inhibitory action against *Leptospires* proved by the screening test, were added. On the repeated experiments, almost no leptospiral growth was observed in the cultures of kidney tissue-pieces in both Korthof's and Stuart's media. Thus, the use of tissue-pieces will certainly lower the isolation rate of *Leptospires*.

It has been experienced that the bacterial contamination (1, 2, 3), pH value (4), and existing antibodies (5) do influence the isolation rate of *Leptospires* from the kidney. Among those influencial factors, contamination is thought most important. However, the source of such contamination has not been fully investigated. If it is originated without the kidney, it can possibly be prevented. In order to avoid such bacterial contamination and to dilute the pH value as well as antibodies, it is a common practice to prepare a series of dilutions of the suspicious sample for an isolation purpose.

On the other hand, as to the isolation of *Leptospires* from the kidney, some investigators (6, 7), without using the tissue emulsion, have directly put small pieces of kidney tissue into the media for the cultivation. This procedure is simple and it can be more adaptable if there is no possibility of contamination. In fact, less complicate procedure can avoid introducing contamination. But no comparison has been made between the isolation rates of using tissue pieces and tissue emulsions.

The author, in the previous report (8), has stated that certain animal sera have inhibitory action on the growth of *Leptospires* and has introduced the screening test (9) for excluding the sera which possess inhibitory factors. The use of the sera with such undesirable property must be avoided since it will lower the isolation rate of the organism.

The object of this study is to find out the factors, except those already known, which will also influence the isolation of *Leptospires* from the kidney. By using *Leptospires* inoculated guinea pigs, the isolation rates of this organism from kidney tissue-emulsions and kidney tissue pieces were compared.

MATERIALS AND METHODS

Media

Both Korthof's and Stuart's media were chosen for the isolation because they

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are of convenience in the examination for a small number of *Leptospires*.

Korthof's medium was prepared in the manner as described in the previous report (8, 9). Stuart's medium used was the product of Difco Co., 90 ml of either kind of basal media was distributed in a 100 ml tissue culture bottle and to which 10 ml of pooled, screening-tested rabbit sera was added. After it was inactivated at 56 C for 60 minutes, 2 ml of that was further distributed in a screw-capped tube (1×12 cm) for use.

The screening test for individual serum was performed as described in the previous report with a slight modification in which the cultivation period was shortened from the original two weeks to one week. The test was performed 5 times and the finding was that 8 out of 30 rabbit sera (26.7%) did not pass the test because of having growth inhibitory action. Johnson and Gary (10) described that some lots of commercial rabbit sera were inadequate for optimal growth of *L. pomona* and could not be used for the preparation of satisfactory control media. This was not a problem in the present study as those individual sera which had strong growth inhibitory effects against *Leptospires* were excluded.

Strain:

L. australis A was used in the present study. It was one of the strains which had been maintained in our laboratory and would certainly cause leptospiremia in guinea pigs.

In the screening test for the rabbit sera, 10 strains comprising 8 serotypes were used. The origin and the serotypes of these strains were the same as those used in the previous report.

Animal inoculation:

20 young guinea pigs, weighing from 160 to 270 gm supplied by local animal dealers, were divided into 2 groups (10 heads in each group). To each guinea pig in either group, 0.1 ml of 2 weeks old culture of *L. australis A* in Korthof's medium was inoculated subcutaneously. Two weeks following the inoculation kidneys were removed from the animals in Group I. Each kidney was cut into about 15 larger pieces and 45 smaller pieces and those tissue pieces were cultured in Korthof's medium. In the meantime, one of the larger pieces was used in the preparation of tissue emulsion with Korthof's basal medium in a Tembroeck's mortar, and from this emulsion dilutions of 1:10, 1:100 and 1:1000 were made. 0.1 ml of each dilution was inoculated into each of the 2 tubes containing Korthof's medium. They were incubated at 28 C for 2 weeks. To the cultures of kidney tissue-pieces in which no leptospiral growth was noticed, 1 loopful of the leptospiral culture in Korthof's medium was inoculated. Such cultures were incubated for another one week and were examined for the growth of organisms afterwards. From the animals in Group II, kidneys were removed from 2 to 3 weeks following the inoculation. As in Group I, larger tissue-pieces and tissue-emulsion were prepared from those kidneys, and they were cultured in both Korthof's and Stuart's media at 28 C for 2 weeks.

RESULTS

The isolation rates of *Leptospires* from the kidney emulsion and kidney tissue-pieces prepared from guinea pigs in Group I and II were compared. As shown in TABLE I and II, there was a prominent difference between them. *Leptospires* were grown in the cultures of 1:0 (Group I) or 1:0 to 1:100 (Group II) dilutions of kidney emulsions prepared from both groups while almost no growth was observed in the cultures of tissue-pieces. The same result was obtained in the 3 weeks cultures.

The finding of interest was that prominent difference on kidney emulsion cultures was noticed between Group I and II. That is, in Group I, rather poor growth was evidenced in 1:0 dilution and no utmost growth was obtained even the cultivation was extended to 3 weeks. On

TABLE I

Growth of ***Leptospires*** *in the cultures of kidney preparations obtained from Group I guinea pigs*

No. of guinea pig	Weight (gm)	Highest temperature (C)	Emulsion								Tissue-pieces only				Tissue-pieces + a loopful of the culture			
			1:0		1:10		1:100		1:1000		Small		Large		Small		Large	
63	220	40.2(5)†	4+	4+*	2+	1+	−	±	−	−	−	−	−	−	3+	2+	1+	1+
64	255	40.9(5)	±	−	−	−	−	−	−	−	−	−	−	−	4+	4+	+	1+
65	200	39.3(4)	±	1+	±	±	−	−	−	−	−	−	−	−	4+	4+	−	−
66	230	40.8(5)	1+*	1+*	−	c	−	−	−	−	±	−	−	−	c	5+	4+	1+
67	220	40.0(5)	4+*	4+*	1+	1+	1+	1+	±	±	−	−	−	−	3+	4+	±	1+
68	270	40.6(5)	1+*	1+	−	±	−	−	−	−	−	−	−	−	4+	3+	−	±
69	160	39.5(5)	Died on the 11th day after inoculation															
70	240	39.7(4)	−	−	−	−	−	−	−	−	−	−	−	−	4+	4+	±	1+
71	245	40.2(4)	1+	1+	±	±	−	−	−	−	±	−	−	−	5+	5+	−	3+
72	270	40.2(3)	1+	3+	−	−	−	−	−	−	−	±	−	−	3+	3+	−	1+

−: No organism in any field.
±: Less than 1 organism per field.
1+: 1-10 organisms per field.
2+: 11-30 organisms per field.
3+: 31 to 100 organisms per field.
4+: More than 100 organisms per field.
5+: The greatest number of organisms per field.
†: Days after inoculation.
*: Lysis.
c: Contamination.

TABLE II

Comparison of the effects of Korthof's and Stuart's media on the cultivations of ***Leptospires*** *from kidney preparations obtained from Group II guinea pigs.*

No. of guinea pig	Weight (gm)	Interval after inoculation (day)	The highest dilution of emulsion evidencing the growth		Large tissue-pieces			
			Korthof	Stuart	Korthof		Stuart	
82	193	14	Died on the 10th day after inoculation.					
83	185	14	1: 10(5+)	1: 10(5+)	1+*	1+	1+	1+*
84	225	14	—	—	—	—	—	—
85	190	14	1: 10(5+)	1: 10(5+)	—	—	—	—
86	190	14	—	—	—	—	—	—
87	175	14	1: 0(5+)	1: 10(5+)	—	—	—	—
88	170	21	1:100(5+)	1: 10(5+)	—	±	—	—
89	200	21	1: 10(4+)	1:100(5+)	—	—	—	—
90	230	21	—	—	—	—	—	—
91	190	21	1: 10(4+)	1:100(3+)	—	±	—	—

*: Lysis.

the contrary, maximum growth was observed in any dilution of 2 weeks cultures in Group II.

Both larger and smaller kidney tissue-pieces obtained from Group I were cultured in Korthof's media and the result, as shown in TABLE I, was that none did evidence the growth of 1+. To each of those cultures, 1 loopful of the leptospiral culture in Korthof's medium was inoculated and, one week later, as shown in TABLE I, the leptospiral growth of 3+ to 5+ was noticed in the smaller tissue-pieces cultures while that of 1+ to 4+ in the others.

As shown in TABLE II, almost the same results were obtained when Korthof's and Stuart's media were used in the cultivation of tissue-emulsion and tissue-pieces. However, the use of Stuart's medium has an advantage of indicating bacterial contamination as phenol red has been added to this medium. When larger tissue-pieces were cultured in Stuart's medium the color of the medium was changed without any contamination, but, in the culture of smaller tissue-pieces such change did not take place.

In Group I, leptospiral lysis was observed in the culture of 1:0 dilution of kidney emulsion, whereas none was seen in the dilutions of higher than 1:10.

DISCUSSION

Guinea pigs were inoculated with *L. australis A* and the kidneys of those animals were harvested 2 to 3 weeks after the inoculation. On the repeated experiments, it has been observed that *Leptospires* can hardly grow in both Korthof's and Stuart's media in which the kidney tissue-pieces, either small or large, were cultured. On the contrary to this finding, leptospiral growth was evidenced in the media to which up to 1:100 dilutions of the kidney emulsion were inoculated. One loopful (3 mm) of 2 weeks old leptospiral culture was inoculated into each one of the cultures of kidney tissue-pieces in which no leptospiral growth took place. The growth of 1+ to 5+ was evidenced after 1 week's cultivation though there was difference regarding the growth between the cultures of larger and smaller tissue-pieces. On the other hand, there was no agglutination lysis taking place in cultures. It is clear from these finding that the failure for *Leptospires* to grow in the cultures of tissue-pieces was not related to the presence of antibodies in the medium. Besides, it is hard to believe that the pH of medium would inhibit the growth of *Leptospires* because there was

no change in the color of the Stuart's medium to which small pieces of kidney tissue were added. The leptospiral growth as such usually stopped at a certain level and further extention of culturing duration would hardly elicit further growth. Though the intrinsic factor of the kidney tissue which inhibited the growth of *Leptospires* can not be made clear at this time, it is conceivable that the *Leptospires* which were blocked in the kidney tissue would probably die out due to the influences of antibodies and acidified pH. Accordingly, on the isolation of *Leptospires* from kidney, the use of kidney tissue-pieces will undoubtedly lower the isolation rate.

As to the leptospiral growth in the cultures of kidney emulsion, a great difference was observed between Groups I and II. That was the growth of 4+ grade was evidenced only in 2 out of 9 guinea pigs in Group I while that of 4+ to 5+ grades was shown in all of the animals in Group II. Though the exact cause of such difference is not known, it is presumable that the difference may have some connection with the feeding.

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