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LEPTOSPIRAL INFECTIONS IN RATS. THE PRESENCE OF SPECIFIC LEPTOSPIRAL IMMUNE BODIES IN THE SERUM AND THEIR RELATIONSHIP TO CARRIER CONDITIONS

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WORKERS in many parts of the world have shown rats and various rodent species to be carriers of *Leptospira*. The observations made in Great Britain are summarized in Table I, from which it is seen that there is a wide variation in the percentage of rats found to harbour *Leptospira* in different localities of this country. The disparity in the incidence rates may, probably, depend on local conditions, and also, to some extent, on the age of the animals examined, since there is some evidence to show that more carriers will be found amongst the fully grown than in the young rodents.

The serological types of leptospira which may be found differ amongst the various species of rodents encountered in different regions of the world (Fletcher, 1927; Taylor & Goyle, 1931; Kaneko *et al.* 1936; Alston & Brown, 1937), but so far in Great Britain there is proof of the existence of only one type, namely, *L. icterohaemorrhagiae*. As regards the presence of immune bodies in the sera of rats, comparatively few observations appear to have been made. Brown & Davis (1927) examined the sera of 100 London rats by means of the adhesion test. They were able to show that thirty-two specimens gave positive reactions with human and rat types of *Leptospira*, four reacted positively with human, rat and Eastern strains, while seven samples gave positive reactions with *L. biflexa*. Langworthy & Moore (1927) mixed the serum of rats with living emulsions of *Leptospira in vitro*, and after an incubation period examined drops of the preparation by dark-ground illumination methods for agglutination and lysis. They found that thirty-five sera out of fifty-five specimens gave a positive reaction. They correlated these findings with the results obtained by inoculation of emulsions of rat kidney into guinea-pigs, and found that in eighteen instances in which the animal test showed the presence of *Leptospira* the sera gave agglutination or lysis when tested against the leptospiral emulsion. In seventeen instances, however, while the sero-reaction was positive, animal inoculation failed to show the presence of virulent *Leptospira*. Since these observations were made the technique of the serological reactions has been greatly improved, and it was thought worth while to reinvestigate the relationship of immune bodies to the presence of *Leptospira* in the kidneys of these animals.

Table I. *Examination of rats for* L. icterohaemorrhagiae *by workers in Great Britain*

Worker	Date	Locality	Rats examined and species	Result of guinea-pig inoculation	Result of microscopic examination	Total percentage positive
Coles	1918	Bournemouth	100 *R. norvegicus*	—	9	9
Foulerton	1919	London	3 *R. rattus* 98 *R. norvegicus*	4	—	4
Stevenson	1922	London	4 *R. rattus* 96 *R. norvegicus*	—	30	30
Smith	1924	Aberdeen	100 *R. norvegicus*	23	—	23
Buchanan	1927	Scotland	2 *R. rattus* 164 *R. norvegicus*	61 by both methods		36·7
Middleton	1929	Oxford	235 *R. norvegicus*	—	98	41·7
Coppinger	1936	Aldershot	58 *R. norvegicus*	12	13	46·4

Methods

Rats were brought to the laboratory, as they were trapped in cages, by the city rat-catchers. They were then transferred to smaller metal boxes, and after being killed by coal gas were autopsied. Sufficient blood was then obtained from the heart for the sero-reaction, and the kidneys were removed and placed in sterile Petri dishes. They were then emulsified in saline, and 2 c.c. of the emulsion injected intraperitoneally into two young guinea-pigs. Direct microscopic examination of the materials was not carried out as a routine. If virulent *Leptospira* were present in the kidney emulsion, the guinea-pigs usually died within 7–12 days after inoculation. The surviving animals were retained until the 16th day, and if, by chance, one animal died of a typical leptospiral infection and the other survived, then the serum of the surviving animal was examined for immune bodies while the kidneys were emulsified and reinoculated into a further pair of young guinea-pigs. In addition, when a positive sero-reaction had been obtained in a rat, and when the guinea-pigs failed to show the presence of *Leptospira*, these animals were killed, their sera tested for leptospiral immune bodies, and their kidneys inoculated into a further pair of animals.

From the guinea-pigs which died of leptospirosis, a small piece of the liver was removed with aseptic precautions, emulsified in a sterile Petri dish in saline, and from this several tubes of Schüffner's leptospiral medium (Smith, 1937) were inoculated and cultured, while dark-ground illumination methods were employed for the demonstration of the *Leptospira*. The sero-reactions on the rat's and guinea-pigs' sera were carried out by the Schüffner technique, which has already been fully described (Davidson *et al.* 1934), and the end-titre of the agglutinins and lysins against cultures of *L. icterohaemorrhagiae* Wijnberg determined.

Results of serological tests and animal inoculation

The positive findings in a series of 117 rats are given in Table II. In sixty-eight rats the sero-reaction was negative in a dilution of 1 : 10, but in four instances *Leptospira* were recovered by guinea-pig inoculation with emulsions

Table II. *Rats. Immune bodies in relation to the presence of* L. icterohaemorrhagiae

No.	Size	Sex	Sero-reaction	Presence of *Leptospira* guinea-pig inoculation A	B
1	F.G.	M	1:100	+	+
2	F.G.	M	1:30	+	−
3	F.G.	F	—	+	−
4	F.G.	M	—	+	−
11	F.G.	M	1:30	+	+
12	F.G.	M	1:30	−	−
13	F.G.	M	1:100	+	+
14	F.G.	F	1:10	+	+
15	F.G.	F	1:100	+	−
16	F.G.	F	1:100	+	+
17	F.G.	M	1:300	+	+
18	F.G.	F	1:1000	+	+
19	F.G.	F	1:30	+	+
21	3/4	M	1:10	+	+
23	F.G.	M	1:30	+	+
24	F.G.	F	1:30	+	+
26	F.G.	M	1:30	−	−
27	F.G.	M	—	+	+
29	3/4	M	1:300	−	−
36	3/4	F	1:300	+	+
48	1/2	F	—	+	−
50	F.G.	M	1:300	−	−
51	3/4	M	1:300	−	−
52	1/2	M	1:300	+	+
53	F.G.	M	1:10	+	+
54	3/4	M	1:100	+	+
56	F.G.	M	1:10	−	−
57	F.G.	F	1:300	−	−
59	F.G.	M	1:100	+	+
68	1/2	M	1:30	+	+
70	F.G.	M	1:100	−	−
71	3/4	F	1:300	−	−
72	3/4	M	1:300	−	−
73	3/4	M	1:300	−	−
74	F.G.	M	1:100	−	−
75	F.G.	M	1:100	+	+
76	1/2	M	1:10	−	−
77	1/2	F	1:10	−	−
78	3/4	F	1:10	−	−
79	3/4	M	1:10	−	−
80	F.G.	F	1:30	−	−
81	F.G.	F	1:30	−	−
84	F.G.	M	1:100	+	+
86	3/4	M	1:10	−	−
90	1/2	F	1:300	−	−
91	1/2	M	1:30	−	−
93	F.G.	M	1:10,000	+	+
97	1/2	M	1:10	+	+
107	F.G.	M	1:100	+	+
108	3/4	M	1:10	+	+
109	F.G.	F	1:300	+	+
111	3/4	F	1:300	+	+
113	3/4	M	1:100	+	+

F.G. = full grown; 3/4 = three-quarters grown; 1/2 = half-grown.

of kidneys. In twenty-one rats the sero-reaction was positive in dilutions ranging from 1 : 10 to 1 : 300, and yet animal inoculation failed to show the presence of *Leptospira*. In twenty-eight instances the agglutination and lysis

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test was positive, and virulent *Leptospira* were recovered by animal inoculation. Thus a total of thirty-two rats, or 27·3%, were shown to be carriers of virulent *Leptospira* compared with 23% of 100 rats examined in 1924.

The relationship of the titre of the immune bodies in the serum to the presence of *Leptospira* was as follows:

	Titre of immune bodies in serum							
	<1 : 10	1 : 10	1 : 30	1 : 100	1 : 300	1 : 1000	1 : 3000	1 : 10,000
Leptospira-positive	4	5	6	10	5	1	0	1
Leptospira-negative	64	6	5	2	8	0	0	0

In sixty-eight animals with an immune body titre of less than 1 : 10, sixty-four failed to show *Leptospira* in their kidneys; in eleven animals with a serum titre of 1 : 10, five positive guinea-pig inoculation tests resulted; in eleven rats with a serum titre of 1 : 30, six showed the presence of virulent *Leptospira*; in twelve animals with a serum titre of 1 : 100, ten were positive on animal inoculation; in thirteen with a titre of 1 : 300, five positive inoculations were obtained; and finally in two rodents in which the serum titre was 1 : 1000 and 1 : 10,000 respectively, *Leptospira* were recovered from the kidneys of both. The number of tests carried out is too small to permit any statistical correlation between the presence of immune bodies and virulent *Leptospira*, but when the sera are positive in the lower dilutions it does not necessarily follow that the animals will be found to harbour virulent *Leptospira*. Presumably a certain number of rodents have been infected at some time previous to examination but have either not developed carrier conditions or, if they have, the *Leptospira* have been eventually eliminated.

In five instances in which one guinea-pig died of typical leptospirosis and the other animal survived, the second guinea-pig was killed between the 16th and 20th days after inoculation, the blood was examined for leptospiral immune bodies, and the kidney emulsions reinoculated into two more guinea-pigs, with the following results:

	Guinea-pig survived	Result of reinoculation of kidneys into 2 further animals	
Original rat no.	Titre of immune bodies in serum	C	D
2	<1 : 10	−	−
3	1 : 100	+	+
4	<1 : 10	−	−
15	1 : 30	+	+
48	<1 : 10	−	−

In certain instances, therefore, it would appear that either the dose of *Leptospira* contained in the kidney emulsion may not be sufficient to infect and kill the animal, or that the guinea-pig may be immune. In two instances the guinea-pigs inoculated originally from the rat kidneys had become passive carriers of virulent *Leptospira*. In order to prove definitely that the guinea-pigs inoculated with emulsions of kidneys from the sero-positive but *Leptospira*-negative rats had not become carriers, the twenty-one surviving pairs were killed. All their sera failed to show the presence of any immune bodies, and

further inoculation of their kidney emulsions into fresh animals failed to detect the presence of any carriers.

Some attempt was made to classify the rodents into four male and female groups according to their size, and to correlate this with the presence of *Leptospira* in the kidneys and immune bodies in the serum. These findings are presented in Table III. From this it is seen that there is a greater incidence of

Table III. *Presence of* Leptospira *and immune bodies in relation to size of rodents*

Sex	Size	*Leptospira*-positive; sero-reaction negative	*Leptospira*-positive; sero-reaction positive	*Leptospira*-negative; sero-reaction positive	*Leptospira*-negative; sero-reaction negative
M.	F.G.	0	12	6	13
M.	3/4	0	3	5	10
M.	1/2	1	3	2	12
M.	1/4	0	0	1	0
F.	F.G.	1	7	3	9
F.	3/4	0	3	2	8
F.	1/2	2	0	2	11
F.	1/4	0	0	0	1
		Totals			
M. and F.	F.G.	1	19	9	22
M. and F.	3/4	0	6	7	18
M. and F.	1/2	3	3	4	23
M. and F.	1/4	0	0	1	1
		4	28	21	64
		Percentage of total in each group			
M. and F.	F.G.	1·9	37·2	17·6	43·1
M. and F.	3/4	0	19·3	22·5	58
M. and F.	1/2	9	9	12·1	69·6
M. and F.	1/4	0	0	50	50
		3·4	23·8	17·9	54·7

leptospiral carriers and positive immune reactions in the more fully grown animals than in the young. In the fully grown group, 56·7 % showed evidence of infection, in the three-quarters grown group 41·8 %, in the half-grown group 30·1 %, while in the group less than half-grown the numbers are too small to arrive at any conclusion.

Thirty-two rat leptospiral strains derived from the infected guinea-pigs were cultured, and all strains were tested against a monovalent rabbit immune serum with a titre of 1 : 30,000. No substantial difference could be found between the antigenic character of any of the strains, as all reacted by the agglutination and lysis test to practically the full titre of the serum. Absorption tests were not attempted.

Results in rats killed sometime prior to examination

It was thought at one time that rats which had been killed and then brought to the laboratory within 24 hr. of death might still show the presence of *Leptospira*. The kidneys of forty-nine such rats were examined by guinea-pig inoculation, and all failed to show the presence of any virulent *Leptospira*. This result is presumably due to the fact that when death occurs the barrier

established by kidney cell membranes between the immune bodies in the serum and the *Leptospira* in the tubules breaks down, and the *Leptospira* are killed off. A similar difficulty arises in fatal human infections. Death is mainly the result of kidney and liver damage, and usually does not occur until after the immune bodies in the serum have begun to develop. The result is that when emulsions of kidneys obtained at post-mortem are inoculated into guinea-pigs the findings are often negative, whereas in the living subject *Leptospira* may be found in the urine from the 12th day onwards. Similar findings have been recorded by Kristensen (1935).

Summary

1. One hundred and seventeen rats have been examined, and thirty-two or 27·3% were found to harbour virulent *Leptospira.*

2. The correlation of carrier conditions with the presence of immune bodies in the serum showed:

(*a*) *Leptospira*-positive, sero-reaction negative, four or 3·4%.

(*b*) *Leptospira*-positive, sero-reaction positive, twenty-eight or 23·8%.

(*c*) *Leptospira*-negative, sero-reaction positive, twenty-one or 17·9%.

(*d*) *Leptospira* negative, sero-reaction negative, sixty-four or 54·7%.

3. The titres of the immune bodies in their sera have been determined. A positive reaction in the lower dilutions of the serum does not necessarily indicate that the rat is a carrier of virulent *Leptospira.*

4. There is evidence to show that there is a greater incidence of carriers amongst fully grown animals than in the young rodents.

5. Rodents which have been killed prior to examination in the laboratory are unsuitable for the determination of the presence of *Leptospira.*

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