PORCINE LEPTOSPIROSIS : NATURAL AND EXPERIMENTAL.

A Thesis for the Degree of Doctor of Philosophy

in the Faculty of Medicine, University of Glasgow,

submitted by:

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SUMMARY.

The agglutination-lysis test for leptospiral infection ha
been applied to 2,773 samples of porcine sera that were obtained fr
various parts of Scotland, England and Northern Ireland. Antibodie
to three leptospiral serotypes have been detected in 1003 (36.2 per
cent.) of those sera. Evidence of infection was forthcoming as fol
to Lepto. canicola in 79 specimens, to Lepto. icterohaemorrhagiae i
412 specimens and to Lepto. pomona in 206 specimens. In 306 sample
positive reactions were observed with more than one serologically
distinct antigen. In case of Lepto. canicola the concentration of
antibodies varied from 1:10 to 1:30,000. Titres to Lepto. ictero-
haemorrhagiae ranged from 1:10 to 1:10,000. Lysis of Lepto. pomona
was observed in dilutions of serum of up to 1:1000. Not any specim
was found to contain antibodies to either Lepto. grippotyphosa or
Lepto. hyos.

Infection by Lepto. canicola was detected by serological
means in five piggeries in the West of Scotland and later confirmed

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by the isolation of the organism from the renal tissues of pigs that were collected from three of those farms. Culture No. 1078 was recovered soon after canicola fever had been diagnosed in a worker employed on the farm.

In a study of the survival in pig kidney of Lepto. canicol it emerged that the organism could survive for twelve days in natura infected material stored at 0-4°C., and for 21 days in artificially infected normal kidney maintained under the same conditions. The persistence of Lepto. canicola in artifically infected urine kept at room temperature appears to be favoured by a level of pH = 6.2-7.4, provided that bacterial contamination does not ensue. Under such conditions leptospirae may be detectable for up to seven days.

The antibody response to infection by Lepto. canicola was studied in 26 piglets, from three to eight weeks of age. Three of the animals served as controls and the remaining 23 were artificially infected with a culture of Lepto. canicola, (Strain No. 35667) which was administered either through the scarified skin or by subcutaneous inoculation.

All the infected animals developed leptospiral antibodies b the individual responses varied considerably. Titres to the homologo (No. 35667) strain of Lepto. canicola extended from 1:1000 to 1:100,0 while those to the heterologous (Aldgate) strain ranged from 1:300 to

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1:30,000.

Cross-reactions with Lepto. icterohaemorrhagiae to dilutions of from 1:10 to 1:300 occurred in the case of twenty piglets.

The sera of four infected animals gave also cross-reactions to Lepto. pomona to titres of from 1:30 to 1:100.

Reactions with Lepto. grippotyphosa or with Lepto. hyos were not encountered.

Throughout the experiment leptospiral antibodies were not found in the serum of any of the control piglets.

Haematological examination revealed that the total white count of infected animals was increased three-to seven-fold.

Five piglets showed an increase of body temperature of up 105°F. or slightly over, while in fifteen animals there was pyrexia 104 and 104.8°F. In three cases the temperature did not differ from that of the control piglets.

A rash, varying in intensity and in duration, was present in all but one of the infected creatures.

In three cases, two of which involved animals only three weeks old, clinical illness of a few days' duration was manifest in diarrhoea, dullness and loss of appetite, and conjunctivitis also was observed in one instance.

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Macroscopical lesions were not detected in the internal organs of piglets killed at different stages of the experiment.

Leptospirae were demonstrated in wet films made from the supernatant fluid of macerated kidneys taken from three of the infe animals.

Leptospiruria was established in the case of ten animals, times which varied from the fourth to the twelfth week of infection.

By means of blood culture, leptospiraemia was shown to ex at four days after infection in the case of fifteen animals and at seven days after infection in three animals.

In the case of twenty of the infected animals, which were killed two to fourteen weeks after infection, Lepto. canicola was recovered from the kidneys immediately after slaughter and again fr those organs after they had been chilled for twelve days or frozen six days.

Attempts to isolate Lepto. canicola from the liver, splee lungs, lymph-nodes and skeletal muscles of infected piglets were no successful.

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CONTENTS.

PART III.

STUDIES ON THE DEVELOPMENT OF ANTIBODIES IN THE SERUM OF PIGLETS EXPERIMENTALLY INFECTED BY *Lepto. canicola*.

EXORDIUM.

The designation, leptospirosis of swine, applies to several contagio-infectious disorders which are attributable to infection by different species of the genus, Leptospira, and which have only recently received particular attention. Leptospirosis has come to be recognised as a disease of world-wide significance mainly through increasing knowledge of its clinical manifestations together with a wider demand for laboratory diagnostic services and an appreciation of the widespread distribution of leptospirae in many species of animals.

In the pig, fourteen serotypes of Leptospira have been reported but infection by Lepto. pomona appears to prevail in many countries of the world (Johnson, 1939; Gsell, 1946; Savino and Rennella, 1944; Guida, 1948; Collier, 1948; Gochenour et al. 1952; Bohl et al. 1954; Bryan, 1954), where the condition is not only of considerable economic import but is also of much significance to public health (Mochtar, 1940; Terskich, 1940; Gsell, 1945; Collier, 1948; Kirschner et al. 1952; Blagoveshchenskaya, 1957).

To natural infection by different species of Leptospira the clinical response of pigs varies quite considerably and a uniform syndrome is rare even when infection is due only to Lepto. pomona.

(a) Infection by Lepto. pomona.

If infection occur prior to mid-gestation, some or all of the sows may abort, notably, during the later stage of pregnancy (Ray, 1952; Boyer, 1952; Bailey, 1953; Csontos et al. 1955; Ferguson et al. 1956; Gualandi, 1959). Abortion, in fact, may be the main clinical feature. Occasionally, however, a litter of full-term but weakly piglets are born alive, only to die soon after birth (Ryley & Simmons, 1954). The sera of aborting sows usually contain a high titre of specific antibodies. In many aborted foetuses, the most outstanding lesion is focal necrosis of the liver and often there is excess of clear, yellowish fluid in the great serous cavities. In non-gravid sows and in older pigs, Lepto. pomona may cause little, if any, clinical abnormality but, at autopsy, the kidneys usually show pronounced macro- and microscopic changes. Depending on the age of the pig and on the duration and severity of infection, greyish-white foci of up to 2 mm. in diameter may be present in the renal cortex and, sometimes, also in the medulla. In other cases, chronic interstitial nephritis may give rise to shrunken kidneys and adherent capsule (Ryley, 1956). In some outbreaks, piglets from infected mothers may fail to thrive and may manifest irregular fever, anorexia, marked weakness, opisthotonos, 'circling'

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movements, convulsions and paralysis. Autopsy of moribund piglets may reveal the presence of petechiae in the visceral organs together with diffuse nephritis (Gochenour et al. 1952). Other workers have observed fever, jaundice, haemoglobinuria, signs of septicaemia and ecchymotic haemorrhages in the lungs and the stomach. During 1950-51, on some Russian collective farms where human workers also were affected, Demyanowa (1954) described a severe outbreak of Lepto. pomona infection in piglets, 3-4 months old, in which loss of appetite, fever, cachexia, staggering gait and icterus were the chief clinical manifestations and several of the animals died. According to some American workers, nursing sows may suffer from "soft mastitis" with agalactia or with production of abnormal milk. In breeding sows, one attack by Lepto. pomona confers a lasting degree of immunity and, usually, the animals may be returned to service. Porcine carriers, too, may constitute sources of infection for other pigs as well as for cattle and man ("Timely Topics", 1953). Lepto. pomona has been isolated from the kidneys and liver of aborted foetuses by Bryan et al. (1953). Detection of greyish-white foci on the cortical surface of porcine kidneys during routine inspection at a meat-packing plant led to the

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discovery of leptospirosis in a large Canadian piggery (Boulanger et al. 1959). Conjunctivitis, weakness of the hindquarters and stiffness of the neck have been noted in artificially infected piglets (Schmid and Giovanella, 1947).

(b) Infection by Lepto. hyos (mitis).

Pigs may be carriers of the organism (Johnson, 1953; Savino and Rennella, 1945-1948a; Kmety, et al. 1956). Often, the only evidence of infection is the presence of specific antibodies in the serum of affected pigs together with a leptospiruria of varying duration (Zacharija, 1951; van Riel and van Riel, 1954; Keast et al. 1956). In naturally infected sows, abortion and breeding difficulties may occur but the organism appears to be less virulent for the pig than Lepto. pomona inasmuch as Tammemagi and Simmons (1958) failed to produce abortion or neonatal losses in the five sows which were used in their experimental work.

(c) Infection by Lepto. icterohaemorrhagiae.

Clinically, this form of infection is more often observed in young pigs, 3-4 weeks old. Affected animals tend to segregate themselves and stand with drooping ears or hide in the bedding but some become prostrate. Fever, anorexia and jaundice may be detectable. At first, yellow areas appear on

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the ears but later extend to all the mucous membranes and to the whole of the integument and, once jaundice develops, recovery seldom ensues (Klarenbeek and Winsser, 1937; Nisbet, 1951; Field and Sellers, 1951; Power, 1951). In a proportion of cases, violet patches may be seen on the abdominal skin. Epizootics of mild character are occasionally encountered. Leptospiral jaundice of adult swine has been noticed during slaughter and is deemed to be a not unimportant source of human infection, particularly, among workers in the abattoir (Sander, 1935; Wagener, 1942; Annual Report on The Health and Medical Services of the State of Queensland for 1940).

Recently, on twenty-two collective farms in the Rostov area of the Ukrainian Republic, Degtyarev (1960) encountered a mixed infection by Lepto. icterohaemorrhagiae, Lepto. pomona and Lepto. tarassovi (presumably, Lepto. hyos). The mortality-rate attained 50 to 90 per cent. in pigs, up to fourteen weeks of age, but was only 2 to 5 per cent. among older stock.

(d) Infection by Lepto. canicola.

Reports of the last few years furnish convincing evidence that healthy pigs are not infrequently carriers of Lepto. canicola. Indirect indication of infection may be

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afforded by the diagnosis of canicola fever in man (Williams et al. 1953; van der Hoeden, 1956; Seiler et al. 1956; Kmety et al. 1956; Coghlan et al. 1957; Michna, 1959a), or by means of a serological survey of pig sera (Michna, 1958). According to Bezdenezhnykh & Kashanova (1956) infection by Lepto. canicola caused heavy losses among young pigs associated with clinical signs of conjunctivitis, fever of up to 41°C (105.8°F) that lasted for 3-5 days together with loss of appetite, occasional icterus and haemoglobinuria. Older animals manifested only cachexia. Van der Hoeden (1956) described Lepto. canicola infection in two herds of swine, in one of which autopsical and histopathological examination revealed the presence of nephritis although the animals were clinically normal. In the second herd, however, young animals suffered from nephritis associated with manifestations of fever, anorexia, marked weakness and convulsions and there was also concurrent infection by Salm. choleraе-suis.

In the last few years the following leptospiral serotypes have been reported from swine: Lepto. autumnalis and Lepto. pyrogenes (Collier, 1948); Lepto. poi (Babudieri, 1949; Salminen, 1956); Lepto. bataviae (Kolochine-Erber & Colombier, 1950; Salminen, 1956); Lepto. grippotyphosa (Zwierz

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et al. 1951; van Riel & van Riel, 1954; Stoll, 1954); Lepto. saxkoebing and Lepto. Rind born (Zaharija, 1951); Lepto. australis (Keast et al. 1956); Lepto. sejroe and Lepto. ballum (Kmety et al. 1956).

Those findings were based either as a result of isolation of the organisms, or in consequence of serological surveys.

The available evidence indicates that Leptospirosis occurs to highest incidence in large piggeries. Subclinical infection by any serotype may remain undetected for a long time during which the urine of carrier animals constitutes a potential source of infection for other pigs and cattle (Peterson, 1951) and affords a definite risk for human attendants as well.

The original object of this work was to determine the incidence of leptospiral infection of pigs in the British Isles. The problem was approached in three ways and the results are presented under the following headings:

Part I. A Serological Survey of Porcine Sera.

Part II. The Isolation of Lepto. canicola from the Renal Tissues of naturally infected Pigs.

Part III. Studies on the Development of Antibodies in the Sera of Young Pigs experimentally infected by Lepto. canicola.

PART I: A SEROLOGICAL SURVEY OF PORCINE SERA.

1. HISTORICAL.

During the past thirty years, or so, reports of serological surveys of pig sera for the presence of leptospiral antibodies have come from almost every part of the world. Lorey (1932) appears to have been the first worker to record the results of an examination of thirty-eight samples, nine of which contained antibodies that were specific for Lepto. icterohaemorrhagiae and occurred to titres ranging from 1:50 to 1:400. In the serum of a pig that had recovered from infection by the same organism, Schüffner (quoted by Klarenbeek and Winsser, 1937) found homologous antibodies at a serum dilution of 1:1000 on the seventeenth day after clinical recovery seemed complete but, when the test was repeated fourteen days later, the titre had fallen to 1:100. On the other hand, in the sera of two young pigs, which Klarenbeek and Winsser had inoculated with macerated guinea-pig tissue containing numerous Lepto. icterohaemorrhagiae and

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which were examined by Schüffner fourteen days later, specific antibody was demonstrable to dilutions of only 1:10 and 1:30, respectively. Johnson (1939), in search of an animal carrier of Lepto. pomona, which organism had been recovered from a human case, tested the sera of farm animals, including those from 250 pigs, which were collected at random in the abattoir at Brisbane, Australia. He found specific agglutinins to be present in 41.5 per cent. of the porcine samples to titres that varied from 1:200 to 1:3000. In a survey of 64 porcine sera, Mochtar (1940) found 26 samples to contain antibodies against Lepto. pomona to titres varying from 1:100 to 1:10,000; four other samples were positive to Lepto. icterohaemorrhagiae to titres of from 1:100 to 1:500, whilst one serum proved positive to Lepto. pyrogenes (Salinem strain) and one possessed antibodies for Lepto. autumnalis (Rachmat strain). In Russia, interesting observations not unimportant from the point of view of public health were published by Terskich (1940) who found in healthy pigs leptospiral antibodies that were identical with those present in human patients recently suffering from an illness that had been clinically diagnosed as swineherd's disease. Those people became ill after they had bathed in a lake in which the

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pigs had been lately washed. A similarly close relationship between swineherd's disease of young piggery workers and butchers in Switzerland and Lepto. pomona infection of pigs was suggested by Gsell (1945) who demonstrated the presence of antibodies to significant titre in pig sera collected from those parts of the country where swineherd's disease prevailed. Of 173 samples of pig serum 65 per cent. were found to be positive to Lepto. pomona in dilutions of up to 1:100 whilst 30 per cent. had titres that exceeded 1:1000. Out of 64 clinically healthy pigs examined by Collier (1948), thirty were positive to Lepto. pomona, four to Lepto. icterohaemorrhagiae, one to Lepto. autumnalis and one to Lepto. pyrogenes. The same author emphasized that out of twenty-five samples of sera derived from human beings with a history of contact with swine thirteen were found to contain antibodies to five different types of leptospira whereas, in the case of persons who did not have any such contact, some hundreds of samples proved negative. Following upon his diagnosis of swineherd's disease among piggery attendants in Israel, Sandler (1949) examined the sera of the pigs and found 48 per cent. of them to be positive for Lepto. pomona. Serological evidence of Lepto. pomona infection of pigs in Italy was supplied by Babudieri (1949) who encountered

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positive reactions in 23 out of 73 samples of serum which he examined. In Czecho-Slovakia, a high incidence of porcine leptospirosis was recorded by Jelinek (1950) who found that 74.1 per cent. of serum samples contained antibodies to Lepto. icterohaemorrhagiae to dilutions of 1:100, or over, but only 45 per cent. of the samples had a titre of 1:1000. Of 74 samples subjected to agglutination-lysis with Lepto. pomona as antigen, 5.4 per cent. reacted positively. Reports based on serological findings from Croatia (Zaharija, 1951) and from Poland (Zwierz et al. 1951) also indicate a high incidence of leptospirosis of pigs in those countries. Boyer (1952) found titres of 1:100,000 to Lepto. pomona antigen in the sera of five sows with a history of abortion but the sera from another group of five sows reacted to a dilution of only 1:8,000. Kolochine-Erber & Colombier (1950) tested the sera of 172 pigs slaughtered in various large abattoirs and found evidence of infection by Lepto. pomona, Lepto. icterohaemorrhagiae, Lepto. grippotyphosa and Lepto. canicola. In another survey of 84 samples, Rossie & Kolochine-Erber (1954/55) demonstrated that only two were negative; of the remainder 25 had a titre of 1:10,000 and three samples were positive in a dilution of 1:100,000. Out of twelve samples of sera procured from

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abattoir employees, four gave a positive reaction and two of them came from individuals who had manifested typical meningitic symptoms. Covaleda & Pumarola (1953) also reported the finding of leptospiral antibodies in the sera of Spanish pigs and regarded a serum dilution of 1:100, or more, as significant of specific infection. Of 1,990 serum samples collected from 26 piggeries, Bryan (1954) found 293, or 14.7 per cent, to react with Lepto. pomona. The final dilutions of serum were 1:10, 1:100, 1:1000 and 1:10,000 and a sample was deemed positive if half, or more, of the leptospirae were agglutinated or were lysed in one or more tubes. Bryan also concluded that, in sows, abortion is to be regarded as the main clinical manifestation of porcine leptospirosis. Stoll (1954) carried out an interesting survey of two groups of pig sera embracing 545 samples of which 302 were collected from clinically healthy animals. Of the latter, fifty samples came from small holdings on which only one pig was maintained and all fifty samples proved negative. In 24 of the remaining 252 samples, however, antibodies to three leptospiral serotypes were present to titres that varied from 1:100 to 1:1,600. The other 243 sera were collected from pigs suffering from swine fever and of this group 46 samples (18.8 per cent.) gave

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evidence of infection by leptospira. Thus, out of a total number of 545 sera, 70 samples (12.8 per cent.) had titres of up to 1:100 and twenty (3.6 per cent.) yielded a titre of 1:400, or over. Stoll regarded as specific only reactions of 1:400, or more. Antibodies to Lepto. pomona were not found in 283 pig sera examined by van Riel & van Riel (1954) but thirteen samples proved positive to three other serotypes. Following a review of the world literature on leptospirosis of pigs, Burnstein and Baker (1954) conducted an investigation that was, in part, serological and, in part biological. Their serological survey included 285 pig sera collected at random from animals killed in a large meat-packing plant, of which samples sixty-three were found to contain leptospiral antibodies. Leptospiral antibodies were also found in all of 600 samples of commercial swine fever antiserum. Biological exploration depended upon experimental inoculation of Lepto. pomona into young pigs with consequent development of interstitial nephritis. In South-East Asia, a high incidence of leptospiral antibodies in the sera of pigs, other domestic animals and man as well was reported by Wisseman et al. (1955). From Australia Ryley (1956) reported serological evidence of infection of pigs by Lepto. pomona and by Lepto. hyos. The

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occurrence of various leptospirae in Finnish pigs was recorded by Salminen (1956) who examined the sera of 406 swine and found that 43 samples reacted with Lepto. hyos, eleven with Lepto. bataviae, nine with Lepto. poi, six with Lepto. pomona and five with Lepto. icterohaemorrhagiae. The first British observations were provided by Seiler et al. (1956) who examined pig sera in connection with an outbreak of canicola fever among piggery workers in the vicinity of Edinburgh and found that those sera reacted only with Lepto. canicola antigen to titres ranging from 1:100 to 1:10,000. Antibodies to Lepto. pomona, Lepto. hyos and to Lepto. biflexa were not demonstrated. Again from Australia, Keast et al. (1956) published the result of a survey of 487 samples of porcine serum which revealed the existence of infection by Lepto. icterohaemorrhagiae, Lepto. grippotyphosa and Lepto. australis as well as by Lepto. pomona and Lepto. hyos. Antibodies to Lepto. icterohaemorrhagiae and to Lepto. canicola were found in 4.2 per cent. of 1,000 samples of pig serum examined by van Wendt (1957) in Sweden. In Italy, Gualandi (1959) demonstrated the presence of Lepto. pomona antibodies in titres of from 1:500 to 1:5,000 in the sera of sows with a history of abortion. Similar antibodies in a range of serum

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dilutions of from 1:10 to 1:1,000,000 were found in Canadian pigs by Boulanger et al. (1959). Out of 950 samples of pig serum collected in the abattoir at Munich, Schneiderhan (1961) found 65 to be positive to Lepto. icterohaemorrhagiae, seventeen to Lepto. grippotyphosa, eight to Lepto. canicola, seven to Lepto. hyos and five to Lepto. pomona.

The preceding references do not include all those that are available but are sufficient to indicate that leptospirosis of pigs is spread widely throughout the world. The available literature also shows that a considerable range of antibody titres has been detected in pig sera to several leptospiral serotypes. A notable feature of the work reviewed is the difference of opinion pertaining to a diagnostic titre that prevails in various parts of the world, which situation is illustrated in Table 1.

TABLE 1. LEPTOSPIRAL ANTIBODIES DEMONSTRATED IN PIG SERA.

No. of sera examined:	No. of positive reactions:	L. canicola	L. ictero-h.	L. grippo-t.	L. hyos	L. pomona	L. pyrogenes	L. autumnalis	L. australis	L. sanrocking	L. sejroe	L. bataviae.	L. poi	L. Mini bern.	Diagnostic titre:	Country	References:
38	9	-	1/50-1/400	-	-	-	-	-	-	-	-	-	-	-	-	Germany.	Levy, H. 1932.
250	103	-	-	-	-	1/20-1/3000	-	-	-	-	-	-	-	-	1/20	Queensland, Australia.	Johnson, D. W. 1939.
64	26	-	1/100-1/500	-	-	1/100-1/10,000	1/100	1/100	-	-	-	-	-	-	-	Batavia.	Hochter, A. 1940.
173	112	-	-	-	-	1/100-1/1000 & over	-	-	-	-	-	-	-	-	1/400	Switzerland.	Gsell, O. 1952.
64	36	-	1/100 & over	-	-	1/100-1/10,000	1/100 & over	1/100 & over	-	-	-	-	-	-	-	Dutch East Indies.	Collier, W. A., 1948.
73	23	-	1/100	-	-	1/100-1/10,000	-	-	-	-	-	-	1/1000	-	-	Italy.	Rubidori, R. 1949.
74	55	-	1/100-1/1000	-	-	1/100-1/1000 & over	-	-	-	-	-	-	-	-	-	Czechoslovakia.	Jelinek, V. 1950.
31	23	-	1/100-1/1000	1/100-1/500	1/1000	1/100-1/1000 & over	-	-	-	1/1000	-	-	-	1/1000	1/100	Croatia.	Zacheria, I. 1951.
92	24	1/100-1/1280	1/200-1/800	1/100-1/800	1/100-1/800	1/100	-	-	-	1/100-1/800	1/50-1/800	-	-	-	-	Poland.	Zwiers, J. et. al. 1951.
10	10	-	-	-	-	1/8000-1/100,000	-	-	-	-	-	-	-	-	-	U.S.A.	Bovey, R. W., 1952.
64	62	-	-	-	-	1/10,000-1/100,000	-	-	-	-	-	-	-	-	-	France.	Rossi, F. & Kolochine-Erber, R., 1954/55.
214	55	-	1/100 & over	-	1/100 & over	1/100-1/1000 & over	-	-	-	-	-	-	-	-	1/100	Spain.	Covaleda, J. & Fumarola, A., 1953.
1990	893	-	-	-	-	1/10-1/10,000	-	-	-	-	-	-	-	-	-	U.S.A.	Bryan, R. M. 1954.
545	70	-	1/100-1/400 & over	1/100-1/800	-	-	-	-	-	-	1/100	-	-	-	1/400	Germany.	Stoll L. 1954.
283	13	1/10-1/100	1/10-1/100	1/10-1/10,000	1/10-1/3000	-	-	-	-	-	-	-	-	-	1/100	Belgian Congo.	Van Riel, J. & van Riel, M. 1954.
1256	312	-	-	-	1/200 & over	1/200 & over	-	-	-	-	-	-	-	-	1/200	Australia.	Sykes, J. W. 1956.
76	46	1/100-1/1000 & over	-	-	-	-	-	-	-	-	-	-	-	-	-	Scotland.	Seiler, M. M., et al. 1956.
287	313	-	1/30-1/300 & over	1/30-1/300 & over	1/30-1/300 & over	1/30-1/300 & over	-	-	1/30-1/300 & over	-	-	-	-	-	1/300	Australia.	Knott, J. C., et al. 1956.
173	86	1/10-1/10,000	-	-	-	-	-	-	-	-	-	-	-	-	1/100	Scotland.	Coghlan, J. D., et al. 1957.
96	58	-	1/200 & over	-	-	1/200-1/2000	-	-	-	-	-	-	-	-	-	Palestine.	Cosen, S. 1953.
200	15	-	1/100-1/200	-	-	1/100-1/12,800	-	-	-	-	-	-	-	-	1/100	U.S.A.	Bohl, E. H., et al. 1958.
191	26	-	1/160-1/320	-	-	-	-	-	-	-	-	-	-	-	1/320	Germany.	Chemnitz, K. 1953.
12	2	-	1/200	1/100	-	-	-	-	-	-	-	-	-	-	-	Switzerland.	Schmid, G. & Giovanella, E. 1947.
205	61	-	1/200	-	-	1/200-1/10,000	-	-	-	-	-	-	-	-	1/200	U.S.A.	Burnstein, T. & Baker, J. A. 1954.
172	62	1/1000	1/100-1/1000	1/100-1/1000	-	1/100-1/100,000	-	-	-	-	-	1/100-1/12,000	-	-	1/100	France.	Kolochine-Erber, R. & Colombier, V. 1955.
100	8	-	1/150-1/200	-	-	1/150-1/6,000	-	-	-	-	-	-	-	-	1/300	New Zealand.	Kirschner, L., et al. 1958.
133	20	1/200-1/2000	-	-	-	-	-	-	-	-	-	-	-	-	-	Palestine.	Van der Hoeden, J., 1956.

The serological survey covered by this thesis is divisible into three parts which accord with the origin of the material concerned. The first part includes 303 samples of serum which were collected at random from animals that were slaughtered in four abattoirs in the West of Scotland. Since antibodies to Lepto. canicola, Lepto. icterohaemorrhagiae and Lepto. pomona were found in an appreciable proportion of these samples (Michna, 1958), it was decided to carry out further studies on blood samples secured during the slaughter of pigs of known origin. Thus, in the second part of the survey, 2,157 samples of pig serum were tested. During the final phase of the survey, material from animals with a history of breeding difficulties were used.

2. MATERIALS AND METHODS.

(A) ANTIGENS.

In addition to Lepto. canicola and Lepto. icterohaemorrhagiae that were already known to exist in this country, I decided to use three other serotypes of which two, namely, Lepto. pomona and Lepto. hyos, have been most commonly mentioned in world literature in association with infection of pigs. Lepto. grippotyphosa was selected as the fifth antigen because it had been reported from several European countries.

From the late Dr. J. C. Broom, of the Wellcome Laboratories, cultures were obtained of:

1. Lepto. icterohaemorrhagiae (Field strain),
2. Lepto. canicola (Aldgate strain),
3. Lepto. grippotyphosa, syn. Lepto. bovis (Bernkopf),
4. Lepto. hyos, syn. Lepto. mitis (Johnson) and
5. Lepto. pomona (Johnson).

The organisms were cultivated in Stuart's (1946) modification of Schnüffer's medium and the procedure adopted to secure a constant supply of satisfactory antigen was as follows: The freshly prepared medium, containing 10 per cent. of suitable rabbit serum, was distributed in 4 ml. amounts into bijou bottles and after inoculation with about 0.5 ml. of the corresponding culture was incubated aerobically at 32°C., for

for/

48 hours. The cultures were then transferred to another incubator, the temperature of which was maintained at 29-30°C., for a further five days. In that way, very satisfactory growth was obtained and could be easily observed when the culture was viewed against a strong electric lamp. After seven days' incubation the antigens were kept in a cupboard at room temperature. Subcultivation was carried out regularly once a week. Only heavily growing and actively motile cultures, 5-9 days old, were used as antigens throughout the whole survey and were subjected to regular control tests against the known standard leptospiral antisera. The latter were prepared in rabbits by Kitaoka's method, (personal communication from the late Dr. J. C. Broom), which involves three intraperitoneal inoculations of living whole cultures into rabbits four months old, at weekly intervals, in doses of 5 ml., 10 ml. and 20 ml., respectively. Within a week after the last inoculation a test bleeding was carried out. When antibody was found to have attained a satisfactory titre, the rabbits were bled out, the sera separated, tested against the homologous as well as heterologous antigens and stored in the refrigerator in 1 ml. amounts. Higher titres of antibodies, namely 1:10,000, were present in both Lepto. canicola and Lepto. icterohaemorrhagiae

icterohaemorrhagiae/

antisera, with slight cross-reaction between these two serotypes, while the remaining three antisera reacted in 1:3,000 dilutions and only with the homologous antigens. Those reactions and the final titres are presented in Table 2.

TABLE 2.

TITRATIONS OF STOCK RABBIT LEPTOSPIRAL ANTISERA.

Rabbit antisera	Antigens:					Final titres
	Lepto. canicola	Lepto. ictero-h.	Lepto. grippo-t.	Lepto. hyos.	Lepto. pomona	
Lepto. canicola	1:10,000	1:100	-	-	-	1:10,000
Lepto. ictero-h.	1:30	1:10,000	-	-	-	1:10,000
Lepto. grippo-t.	-	-	1:3000	-	-	1:3000
Lepto. hyos.	-	-	-	1:3000	-	1:3000
Lepto. pomona	-	-	-	-	1:3000	1:3000

(B) THE SURVEY SERA.

It was considered probable that more information on the incidence of porcine leptospirosis could be gained by a survey of the sera of pigs coming for slaughter into different abattoirs, since thereby animals from a wider area of the country would be represented. In the first stage of this investigation blood samples were collected at random from pigs slaughtered in four abattoirs in the West of Scotland, namely: Glasgow, Hamilton, Greenock and Paisley. Accordingly, blood was taken from every fifth or tenth pig killed on a particular day and the total number of sera thus obtained was 303.

Sera removed from the clotted blood were stored usually for a few days in the frozen state before they were tested. The technique applied for routine testing was that of Schüffner, described by Wolff (1954). In sterile bell-mouth tubes, 5.5 cm. long by 1.0 cm. in diameter, dilutions of sera were made in sterile normal saline solution of the series, 1:5, 1:15, 1:50 and 1:150. For the first test, which was a screening procedure, three drops of the four dilutions of each serum were prepared for each antigen. The same volume of the corresponding antigen was added to each tube, thus yielding

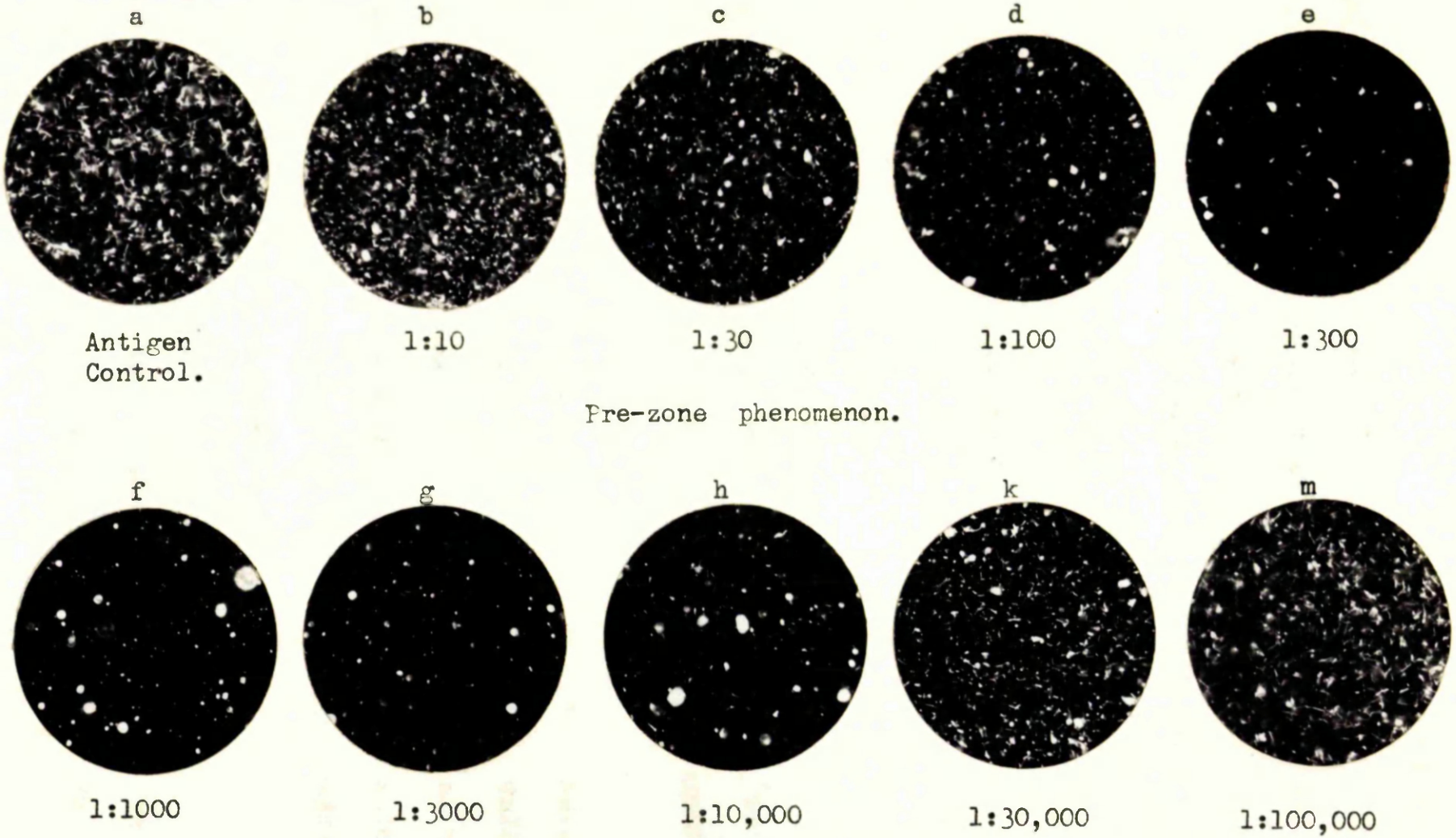


Fig. 1. Agglutination-lysis Test on pig serum containing Lepto. canicola antibodies.

yielding/

final dilutions of serum of 1:10, 1:30, 1:100 and 1:300. The mixtures were shaken thoroughly and left in the incubator at 32°C. for four hours. One drop from each tube was then examined for agglutination-lysis by means of dark-field microscopy, with the aid of a 16 mm. objective and a 20x ocular.

A serum was deemed to be positive when complete lysis of leptospirae occurred in lower dilutions (1:10 and 1:30) or if lysis of approximately 50 per cent., or more, of the organisms took place in the higher dilutions (1:100 or over). A sample was accepted as positive only if such a result obtained at a second test. If the need arose, further dilutions of serum were prepared. The photographs presented in Fig. 1 illustrate the appearance of agglutination-lysis in different dilutions of pig serum positive to Lepto. canicola, as observed by low power dark-ground microscopy. Thus, Fig. a illustrates the density of the Lepto. canicola antigen that was used as a control during the reading of the tests. The persistence, but gradual diminution in the number of live and motile leptospirae in Figures b, c and d denotes the so-called "pre-zone phenomenon" which is almost always encountered in the lower dilutions of sera that show a high titre of leptospiral antibodies.

antibodies./

Complete lysis is a feature of Figures e, f and g (dilutions of 1:300, 1:1000 and 1:3,000, respectively), whereas a small number of organisms occur in Fig. h and many more are present in Fig. k (dilutions 1:10,000 and 1:30,000, respectively). There is little, if any, optical difference between Fig. m and a. Since the density of the antigen in Fig. k appears to be about half of the control, 1:30,000 is regarded as the titre of the serum under discussion.

TABLE 3.

LEPTOSPIRAL ANTIBODIES FOUND IN THE SERA OF PIGS

COLLECTED AT RANDOM IN FOUR ABATTOIRS IN THE WEST OF SCOTLAND.

Source of serum samples	Total No. examined	Negative	Positive to Lepto:				Total positive	Per cent.
			canicola	ictero-h.	pomona	Mixed reactions		
Glasgow	154	54	-	56	13	31	100	64.9
Hamilton	43	19	-	12	3	9	24	55.8
Paisley	45	23	4	12	3	3	22	48.9
Greenock	61	16	-	22	4	19	45	73.8
Total:	303	112	4	102	23	62	191	63.0

3. RESULTS.

Out of 303 samples of pig serum, antibodies to three leptospiral serotypes were demonstrated in 191, (63.0 per cent). Antibodies to Lepto. canicola were present in four of the samples. Another 102 sera proved positive only to Lepto. icterohaemorrhagiae and 62 samples gave mixed reactions either with Lepto. icterohaemorrhagiae and Lepto. pomona or with Lepto. canicola and Lepto. pomona. In 23 specimens of serum, antibodies to Lepto. pomona alone were found. The highest incidence of leptospiral antibodies was observed in samples received from Greenock abattoir, since 45 out of 61 sera, (73.8 per cent.) proved positive to Lepto. icterohaemorrhagiae or to Lepto. pomona, or to both antigens. Out of 154 samples collected from the Glasgow Meat Market 100 (64.9 per cent.) proved similarly positive as did also 24 out of 43 samples (55.8 per cent.) obtained from Hamilton. From Paisley abattoir 22 out of 45 samples (48.9 per cent.) proved positive and were the only sera in which antibodies to all three leptospiral serotypes were demonstrated. Not any sample was found to contain antibodies to Lepto. grippo-typhosa or to Lepto. hyos. Those reactions are summarised in Table 3.

TABLE 4.

THE DISTRIBUTION OF LEPTOSPIRAL ANTIBODIES IN 303 PIG SERA EXAMINED.

Antigen:	Serum dilutions:							Total:	Percentage:
	1:10	1:30	1:100	1:300	1:1000	1:3000	1:10,000		
Lepto. canicola	-	-	1	1	2	-	1	5	1.6
Lepto. ictero-h.	9	58	69	21	4	2	1	164	54.1
Lepto. pomona	4	51	28	1	1	-	-	85	28.1

The overall high incidence may be explained by the fact that reactions were encountered in all the serum dilutions employed. Though rare in occurrence (1.6 per cent.), Lepto. canicola antibodies appeared to be in high concentration since only two samples had titres of 1:100 and 1:300 respectively, while another two had 1:1000 and one had 1:10,000. In contrast, most of the positive reactions to Lepto. icterohaemorrhagiae antigen were observed in the lower serum dilutions and only seven sera were found to contain antibodies in titres ranging from 1:1000 to 1:10,000. Altogether 164 samples (54.1 per cent.) reacted positively with that pathogen. In 85 samples (28.1 per cent.) lysis of Lepto. pomona took place, but there was only one serum with a titre of 1:1000 and another with a titre of 1:300, whilst in the remaining 83 sera the titre varied from 1:10 to 1:100. Table 4 illustrates those reactions.

Those preliminary findings are regarded as of medical as well as of veterinary significance. The apparently high incidence of infection of pigs by Lepto. icterohaemorrhagiae is attributable to the fact that the common rat (Rattus norvegicus), a recognised carrier of the pathogen, is only too often found in piggeries, where food and bedding become contaminated by rodent urine containing the causal organism of Weil's disease. The finding of high titres of Lepto. canicola antibodies in three out of 303 samples and the demonstration of low titres in another two sera furnishes additional evidence of the existence of that type of leptospiral infection in pigs of this country, and confirms previous reports by Seiler et al. (1956) and Coghlan et al. (1957).

Considerable interest attaches to the observations that (a) twenty-three samples reacted positively with Lepto. pomona antigen alone in titres ranging from 1:10 to 1:1000 and (b) an additional 62 sera cross-reacted with other antigens. Since it is generally believed that the agglutination-lysis test for leptospirosis has a high degree of specificity and that, once antibodies appear in the serum of the host where they persist for some time after infection, any titre constitutes strongly presumptive evidence of past or of

of/

present infection.

An attempt to clarify the latter question led to the second phase of the serological survey during which further studies were made on samples of sera taken from pigs in diverse parts of the country. As far as possible, the addresses of origin of the samples were obtained so that, when positive serum reactions were encountered, not only were additional blood samples sought but kidneys from pigs reared on the farm were also solicited for the purpose of isolation of leptospira. Altogether, 2,157 sera were collected from animals slaughtered in Scotland, England and N. Ireland. The samples from Scottish pigs were derived from stock reared in the counties of: Argyll, Ayr, Dunbarton, Dumfries, Kinross, Lanark, Orkney, Peebles, Perth, Renfrew and Stirling and amounted to 1105 specimens, representative of 160 piggeries. Among Scottish pigs, leptospiral antibodies were demonstrated in 485 sera (43.9 per cent.), whilst the remaining 620 sera proved negative. The detailed results with the various antigens are given in Table 5. As a result of the survey the presence of infection of pigs by Lepto. canicola was established on five farms located within, approximately, 10 miles of Glasgow. On three of the farms,

TABLE 5.

LEPTOSPIRAL ANTIBODIES IN PIG SERA COLLECTED IN SCOTTISH, ENGLISH AND N. IRISH ABATTOIRS.

Abattoir	Number examined:	Negative	Positive to:				Total	Percentage
			Lepto. canicola	Lepto. ictero-h.	Lepto. pomona	Mixed		
Scotland	1105	620	75	121	142	147	485	43.9
England	578	438	-	78	11	51	140	24.2
N. Ireland	474	399	-	69	-	6	75	15.8
Total:	1157	1457	75	268	153	204	700	32.5

farms,/

infection was subsequently confirmed by isolation of the organism from the kidneys of resident animals. In one instance, recovery of the organism took place some time after the occurrence of canicola fever in a piggery worker (strain 1078, Michna, 1959a). Of 578 English samples obtained from Cambridgeshire, South Devon and Staffordshire, not one was found to contain antibodies to Lepto. canicola. On the other hand, out of 140 positive reactions (24.2 per cent.) 78 concerned Lepto. icterohaemorrhagiae, eleven involved Lepto. pomona and 51 lysed both antigens. The remaining 438 samples were negative.

From 45 farms in N. Ireland 474 samples of serum were submitted. Leptospiral antibodies were found in 75 (15.8 per cent.) of the samples, of which 69 reacted with Lepto. icterohaemorrhagiae alone, whilst the other 6 gave some cross-reaction with Lepto. pomona as well. Again not any reaction with Lepto. canicola occurred, nor were any of the sera found to contain antibodies to Lepto. grippotyphosa or to Lepto. hyos. The residual 399 samples proved negative for leptospira.

The results of the survey are summarized in Table 5.

TABLE 6.

THE DISTRIBUTION OF LEPTOSPIRAL ANTIBODIES IN THE SERA OF SCOTTISH PIGS FROM KNOWN PREMISES.

Antigens:	Serum dilutions:								Total Positive	Percentage
	1:10	1:30	1:100	1:300	1:1000	1:3000	1:10,000	1:30,000		
Lepto. canicola	1	5	15	12	18	18	11	4	84	7.6
Lepto. ictero-h.	27	83	106	23	8	1	-	-	248	22.4
Lepto. pomona	55	141	77	12	2	-	-	-	287	25.9

The sera of Scottish pigs presented titres of leptospiral antibodies that varied considerably. There was only one sample that contained Lepto. canicola antibodies in 1:10 dilution, other five had a titre of 1:30, fifteen reacted at a dilution of 1:100, while twelve sera had a titre 1:300; eighteen reacted to 1:1000 and another eighteen to 1:3,000. Eleven sera had a titre of 1:10,000 and four reacted at a dilution of 1:30,000. Of 84 samples of sera which proved positive to Lepto. canicola, 63 reacted in titres ranging from 1:300 to 1:30,000 and in 21 samples only did the titre of antibodies fall below 1:300. In contrast only 32 sera, representing but a small proportion of those positive to Lepto. icterohaemorrhagiae antigen, contained homologous antibodies in high concentration and, indeed, a titre of 1:3,000 was obtained with one sample whilst eight sera reacted at 1:1000 and twenty-three at 1:300. The titres of the remaining 216 sera ranged from 1:10 to 1:100. Lepto. pomona antibodies also occurred to low concentration in the majority of the samples tested. Thus only two sera had titres of 1:1000, twelve reacted at a dilution of 1:300, seventy-seven at 1:100, one hundred and forty-one at 1:30 and fifty-five samples reacted at 1:10. Detailed figures of the distribution of leptospiral antibodies in sera from Scottish pigs are given in Table 6.

TABLE 7.

TITRES OF ANTIBODIES IN SERA OF ENGLISH PIGS.

Antigens:	Serum dilutions:							Total
	1:10	1:30	1:100	1:300	1:1000	1:3000	1:10,000	
Lepto. ictero-h.	15	52	47	11	3	-	1	129
Lepto. pomona	10	27	22	3	-	-	-	62

Out of 578 serum samples from English pigs one contained antibodies to Lepto. icterohaemorrhagiae to a dilution of 1:10,000; three had titres of 1:1000, eleven of 1:300 and forty-seven of 1:100; fifty-two sera reacted at 1:30 and fifteen gave the lowest titre of 1:10. Similarly positive reactions with Lepto. pomona antigen occurred in the lower serum dilutions, namely ten sera had a titre of 1:10, twenty-seven samples had 1:30, other twenty-two reacted at 1:100 and only three lysed this antigen to a dilution of 1:300. Thus, with the exception of 18 samples fifteen of which reacted positively to Lepto. icterohaemorrhagiae at dilutions of from 1:300 to 1:10,000 and three to Lepto. pomona at 1:300, the remaining sera contained antibodies to titres of only 1:10 to 1:100. Those figures are summarized in Table 7.

TABLE 8.

TITRES OF ANTIBODIES IN SERA OF IRISH PIGS.

Antigens:	Serum dilutions:							Total
	1:10	1:30	1:100	1:300	1:1000	1:3000	1:10,000	
Lepto. ictero-h.	-	6	35	14	17	2	1	75
Lepto. pomona	-	4	1	-	-	-	-	5

Examination of samples of serum from Northern Ireland revealed a relatively low incidence of porcine leptospirosis, which was associated with infection only by Lepto. icterohaemorrhagiae. Six sera showed a titre of 1:30, thirty-five of 1:100, fourteen of 1:300; seventeen samples reacted at 1:1000 and two at 1:3,000, but only one reached a titre of 1:10,000. Five samples gave mixed reaction with Lepto. pomona as well, at dilutions of 1:30 and 1:100, but such a result may be indicative of the so-called co-reaction phenomenon. The concentration of Lepto. icterohaemorrhagiae antibodies was rather higher than that encountered in English pigs and, indeed, in nearly 50 per cent. of the samples the titres ranged from 1:300 to 1:10,000, as shown in Table 8.

TABLE 9.

AGGLUTINATION-LYSIS TESTS OF SERA

FROM PIGS WITH A HISTORY OF BREEDING DIFFICULTIES.

Total No. examined	Negative	Positive to Lepto.					Percent.
		canicola	ictero-h.	pomona	Mixed	Total	
313	201	-	42	30	40	112	35.8

In Great Britain, abortion together with neonatal mortality and other breeding difficulties occur to quite high incidence, especially in some large piggeries, and so constitute matters of considerable economic importance. The possibility that those anomalies may be of leptospiral origin was responsible for the final stage of the survey, which was designed to include samples of serum derived only from animals with such histories. Most of the samples were sent from England but some came from Scotland and several were obtained from Northern Ireland. In all cases previous investigation for brucellosis by other workers proved negative. Of 313 specimens examined 112 (35.8 per cent) yielded positive reactions. Such a figure is rather higher than that returned by random English samples but is lower than the precentage experienced with similar sera from Scottish pigs. Forty-two specimens were found to contain Lepto. icterohaemorrhagiae antibodies, thirty sera lysed Lepto. pomona antigen alone and forty others reacted with both antigens. Not any sample of the group showed evidence of infection by Lepto. canicola and 201 sera proved entirely negative. Those results are presented in Table 9.

TABLE 10.

TITRES OF ANTIBODIES IN SERA FROM ANIMALS WITH BREEDING DIFFICULTIES.

Antigens:	Serum dilutions:							Total
	1:10	1:30	1:100	1:300	1:1000	1:3,000	1:10,000	
Lepto. ictero-h.	5	10	39	13	11	3	1	82
Lepto. pomona	4	15	45	6	-	-	-	70

In the group of sera from animals with breeding difficulties, the distribution of antibodies differs little from that already experienced although higher titres to Lepto. icterohaemorrhagiae were observed. One serum reacted to dilutions of up to 1:10,000, three were positive at 1:3,000, eleven sera had titres of 1:1000 and thirteen reacted at a dilution of 1:300. In the remaining fifty-four samples, Lepto. icterohaemorrhagiae antibodies were demonstrable in dilutions ranging from 1:10 to 1:100. Only six sera contained antibodies to Lepto. pomona in dilutions of up to 1:300, whilst sixty-four samples gave low titres of from 1:10 to 1:100, as shown in Table 10.

TABLE 11.

RESULT OF A SEROLOGICAL SURVEY OF 2,773 SAMPLES OF PIG SERA.

Total No. examined	Negative	Positive to:							Percent:
		Lepto. canicola	Lepto. ictero-h.	Lepto. grippo-t.	Lepto. hyos.	Lepto. pomona	Mixed	Total	
2,773	1,770	79	412	-	-	206	306	1003	36.2

The full survey involved 3 groups of porcine sera, and covered a total of 2,773 samples, of which 1,003 (36.2 per cent.) proved to contain leptospiral antibodies. Seventy-nine samples (2.9 per cent.) were positive to Lepto. canicola, 412 sera (14.8 per cent.) reacted with Lepto. icterohaemorrhagiae per se and 206 (7.4 per cent.) with Lepto. pomona antigen alone. Three hundred and six samples of serum (11 per cent.) contained antibodies to more than one antigenically unrelated serotype, that is, either to Lepto. icterohaemorrhagiae and Lepto. pomona or to Lepto. canicola Lepto. pomona. Not a single specimen was found to react with either Lepto. grippotyphosa or Lepto. hyos. The remaining 1,770 sera (63.8 per cent.) were negative. The results of the whole survey are summarised in Table 11.

TABLE 12.

THE DISTRIBUTION OF LEPTOSPIRAL ANTIBODIES IN ALL SERA EXAMINED.

Antigens:	Serum dilutions:								Total
	1:10	1:30	1:100	1:300	1:1000	1:3000	1:10,000	1:30,000	
Lepto. canicola	1	5	16	13	20	18	12	4	89
Lepto. ictero-h.	56	209	296	82	43	8	4	-	698
Lepto. pomona	74	238	173	22	3	-	-	-	510

It further emerged that most of the sera positive to Lepto. canicola contained specific antibodies in relatively high concentration, since four samples had titres of 1:30,000, twelve reacted at a dilution of 1:10,000, eighteen at 1:3,000, twenty at 1:1000 and thirteen at 1:300. Thus, sixty-seven sera (75.3 per cent.) contained Lepto. canicola antibodies in titres ranging from 1:300 to 1:30,000, whilst in twenty-two samples (24.7 per cent.) similar antibodies occurred at dilutions of from 1:10 to 1:100. Of those sera ten gave cross-reaction with Lepto. pomona antigen albeit only at dilutions of 1:10 and 1:30. In 137 sera (19.7 per cent.) Lepto. icterohaemorrhagiae antibodies were found to titres ranging from 1:300 to 1:10,000(vide, Table 12). Titres of from 1:10 to 1:100 were recorded in the case of the remaining 561 samples and represent 80.3 per cent. of the positive reactions which were experienced with Lepto. icterohaemorrhagiae. Only three samples of serum contained antibodies to Lepto. pomona that were detectable in dilutions as high as 1:1000. Other 22 samples yielded a titre of 1:300, whilst the remaining 485 sera (95.1 per cent.) reacted only to dilutions of 1:10 to 1:100.

1:100./

Table 12 illustrates the wide range of titres of leptospiral antibodies that was encountered throughout the survey.

4. DISCUSSION.

The results of the survey pose certain questions. For instance, what significance attaches to the demonstration of leptospiral antibodies in a sample of serum? Do such antibodies indicate specific infections? If so, what titre is to be regarded as indicative of infection? And are results with pig sera capable of the same interpretation as is made of those from other animals.

Bernkopf (quoted by Zaharija, 1951) maintained that in uninfected animals the titre of leptospiral antibodies does not exceed 1:20. In their survey of dog sera Broom and McIntyre (1948) stated: "It is generally agreed that specific agglutination of leptospirae by the serum does not occur in the absence of infection, present or past, and that agglutination, even if present only in low dilution, is proof of such infection." In respect of human sera Broom (1948) declared that a titre of 1:1000, or more, is confirmatory of a clinical diagnosis, whereas one of 1:100 or 1:300 may indicate the presence only of residual antibodies. Little and Baker (1950) considered a titre of 1:20 to be of doubtful import and one of

of/

1:200 to constitute definite evidence of leptospirosis in cattle. According to Zaharija (1951) the standard of interpretation adopted by The Laboratory at St. Gallen, Switzerland, recognises a titre of 1:400 as indicative of an established leptospirosis, whilst a lower titre may denote either incipient or past infection. In respect of horse serum, Heusser et al. (1948) deemed a titre of 1:400 to denote infection, whereas Rimpau (1950) gave the same interpretation to a dilution of 1:1000. If similar criteria be applied to pig sera the results of may work suggest that not one of the 2,773 animals involved had been in contact with either Lepto. grippotyphosa, or Lepto. hyos, and that, in fact, British pigs have been as yet exposed to infection by only three leptospiral serotypes. Infection by Lepto. icterohaemorrhagiae was demonstrated by Nisbett (1951) and by Field and Sellers (1951) when they isolated the organism. The presence of Lepto. canicola antibodies in pig sera has been established by Seiler et al. (1956) and by Michna (1958) and the latter worker (1959a) also recovered the organism from pig kidneys. The existence of infection by Lepto. pomona was implied by Michna (1958) when he reported the finding of homologous antibodies in the sera of 23 pigs. When the late Dr. J. C. Broom examined three of those sera, sent to him

him/

3 months after collection, he reported "These results are certainly very suggestive of infection with Lepto. pomona, and I think it would be well worthwhile making a determined effort to try and isolate strains from the kidneys. This would be providing irrefutable evidence of the presence of Lepto. pomona infection in this country and I think it would be advisable to obtain it since there has so far never been any indication of its presence".

An extensive review of the available literature relating to serological surveys of leptospiral infection clearly indicates that porcine leptospirosis, especially that due to infection by Lepto. pomona is a vexed question in many parts of the world. In all, antibodies to 13 leptospiral serotypes have been detected in pig sera to titres that have varied from 1:10 to 1:100,000, as shown in Table 1. Among the various authorities there is a striking lack of unanimity in regard to the titre that denotes infection and, indeed, almost half of the workers have not expressed any opinion on this important matter. The titres quoted by others have varied from 1:20 to 1:400 and often appear to have been arbitrarily selected without any scientific support. Johnson (1939) took a reaction in a serum dilution of 1:20 to indicate infection.

infection./

According to Zaharija (1951) a titre of 1:100 is evidence of residual antibodies, but this report refers to a small number of pig sera in which antibodies to six different serotypes occurred. Six months after an outbreak of Lepto. icterohaemorrhagiae infection on a farm, Nisbet (1951) found homologous titres to vary from 1:30 to 1:100 in the sera of seven pigs whilst other two animals gave a negative result; not one of the sera reacted with Lepto. pomona antigen. Schneiderhan (1961) examined the sera of sows with a history of abortion and found Lepto. icterohaemorrhagiae antibodies in dilutions ranging from 1:100 to 1:1,600; attempts to recover the pathogen failed. Gsell (1952) maintained that the demonstration of specific antibodies in the serum of a host provides speedier evidence of infection than does isolation of leptospira. As proof of infection he took a reaction at a dilution of 1:400 in the case of serum and of 1:4 in respect of spinal fluid but suggested that lower titres may be significant in relation to very recent or to past infection and regarded serum titres 1:100 and 1:200 with caution. In the same series of observations cross-reactions between Lepto. pomona and Lepto. sejroe were only too common and in one instance the serum of a pig breeder tested on the seventh day of illness, reacted

reacted/

with Lepto. pomona at a dilution of 1:100 and with Lepto. sejroe at 1:1,600; 41 days later the serum contained Lepto. pomona antibodies to a titre of 1:25,000, but reacted with Lepto. sejroe to a dilution of only 1:400. In a study of the incidence of Lepto. pomona infection in New Zealand, Kirschner et al. (1952) expressed the opinion that in both human and pig sera the titre of antibodies to Lepto. pomona is usually lower than that to Lepto. icterohaemorrhagiae, because of the milder nature of infection by the former organism, but indicated that the agglutinating titre to both organisms may be identical during the early stage of the disease. In the case of pig serum they accepted a titre of 1:300 to indicate infection. Wolff (1954) stated: "In screening a group of humans or animals for the presence of leptospiral antibodies, positive titres of 1:300 and higher for different strains may have diagnostic importance".

Of 306 sera in the present survey that gave mixed reactions, 296 lysed both Lepto. icterohaemorrhagiae and Lepto. pomona. Two of those samples had a titre 1:1000 to Lepto. pomona but reacted to only 1:30 and 1:100 with the agent of Weil's disease. Another 34 sera containing Lepto. pomona antibodies to a dilution of 1:300 also lysed Lepto. ictero-

icterо-/

haemorrhagiae but only to dilutions of up to 1:100. Among the remaining 260 sera titres were encountered of up to 1:100 and in many samples the titre to both organisms was identical. Only ten sera positive to Lepto. canicola reacted with Lepto. pomona as well, the respective titres being up to 1:10,000 and up to 1:30.

Lepto. canicola, Lepto. icterohaemorrhagiae and Lepto. pomona are antigenically distinct. Cross-reaction between the two former serotypes is usual and may occur to a very limited extent between the two latter organisms. On the other hand cross-reaction between Lepto. canicola and Lepto. pomona has not been encountered (Wolff and Broom, 1954).

In the current survey cross-reaction between Lepto. icterohaemorrhagiae and Lepto. canicola occasionally occurred to the same titre, notably in the case of low dilutions of serum, and is attributable to the close antigenic relationship of the two organisms. According to Gispen and Schuffner, (1959a), non-specific reactions are more common when the infecting organism is the incomplete, or "A", antigenic type of Lepto. icterohaemorrhagiae than when the complete, or "AB", type is involved. Such reactions are most

most/

likely to occur in the early stage of the disease. Thus, Broom (1948) tested a sample of human serum and found that on the 16th day of illness it reacted almost equally to both Lepto. icterohaemorrhagiae and Lepto. canicola but by the 26th day a much higher titre to the former pathogen was forthcoming. Subsequent tests by absorption proved the case to be one of Weil's disease. The interpretation of serological reactions became even more confused when Galton et al. (1956) postulated the theory of "paradoxical reaction". The latter may be defined as the development to higher concentration of agglutinins for a heterologous serotype. Paradoxical reactions have been noted in studies of both human and bovine leptospirosis, for example, Lepto. pomona has been isolated from herds of cattle in which antibodies to Lepto. sejroe mainly were demonstrated. As those serotypes are antigenically distinct, such a finding is difficult to explain, unless one accepts the unlikely assumption that the herd had been originally infected by Lepto. sejroe with consequent development of specific antibodies, and subsequent infection by Lepto. pomona took place.

In respect of Lepto. pomona infection of pigs a more rigid interpretation of serological reactions is

is/

practised by the College of Veterinary Medicine at the University of Illinois (Timely Topics, 1956). A positive reading at any dilution from 1:100 to 1:10,000 is considered to be highly suggestive of infection. A reaction at a dilution of 1:10 is interpreted as doubtful one, in which case the animal is subject to another test in 30 days, when a rise of titre is deemed to indicate advancing infection, whereas convalescence is denoted by a stationary titre, or by a loss of titre. Broom (1959) stated: "In serological surveys titres of 1:100 or more are accepted as significant by most workers, and it is usual to find that the serum reacts with only a single serotype, or a few closely related ones". Stoenner (1957) is of the opinion that the diagnosis of infection by a particular serotype cannot be based on serological findings alone and declared that the leptospira should be isolated and its antigenic composition determined. In reference to small wild rodent in Scotland, Broom & Coghlan (1958) concluded that serological surveys may give a misleading picture of the incidence of leptospirosis inasmuch as only three out of fifteen of those hosts were found to contain antibodies in their sera. Broom et al. (1960) recovered Lepto. bratislava from the kidneys of a hedgehog,

hedgehog, /

the serum of which animal did not possess any homologous antibodies although it reacted with six other antigens to the following dilutions: with Lepto. icterohaemorrhagiae, Lepto. pomona, Lepto. ballum and Lepto grippotyphosa to 1:30 and with Lepto. sejroe and Lepto. bataviae to 1:100.

The references cited and the results obtained during the current survey, therefore, seem to warrant the view that a titre of antibody of 1:300, or over, given by pig serum to a single leptospiral serotype is indicative of infection. In cases of mixed reactions, especially those in which antibodies are demonstrable to a dilution below 1:300, a definite diagnosis, based on a single test cannot be given. It can be seen in Table 1 how many authors reported their findings without any attempt to interpret the results.

The precise significance attachable to titres of antibody encountered in porcine leptospirosis was the aim of further studies involving, firstly, the isolation of leptospires from the kidneys of animals exhibiting Lepto. canicola antibodies in their sera and, secondly, observations on the development and the behaviour of leptospiral antibodies in sera of piglets artifically infected by Lepto. canicola. The results of those investigations are presented in Parts 2 and 3, respectively, of the thesis.

5. SUMMARY.

1. The agglutination-lysis test for leptospiral infection has been applied to 2,773 samples of porcine sera that were obtained from various parts of Scotland, England and Northern Ireland.

2. Antibodies to three leptospiral serotypes have been detected in 1003 (36.2 per cent.) of those sera.

3. Evidence of infection was forthcoming as follows: to Lepto. canicola in 79 (2.8 per cent.) specimens to Lepto. icterohaemorrhagiae in 412 (14.8 per cent.) specimens and to Lepto. pomona in 206 (7.4 per cent.) specimens.

4. In 306 (11.0 per cent.) samples positive reactions were observed with more than one serologically distinct antigen.

5. In the case of Lepto. canicola the concentration of antibodies varied from 1:10 to 1:30,000.

6. Titres to Lepto. icterohaemorrhagiae ranged from 1:10 to 1:10,000.

7. Lysis of Lepto. pomona was observed in dilutions of serum of up to 1:1000.

8. Not any specimen was found to contain antibodies to either Lepto. grippotyphosa or Lepto. hyos.

hyos./

9. All the blood samples positive to Lepto. canicola were obtained from Scottish pigs.

10. Infection by Lepto. canicola was detected by serological means in five piggeries in the West of Scotland and later confirmed by isolation of the organism from the renal tissues of pigs that were collected from three of those farms.

11. The infected herds were situated from a few to several miles apart and contact between the animals was not established.

12. The proportion of positive reactions to Lepto. icterohaemorrhagiae and to Lepto. pomona was highest in sera from pigs reared in Scotland and lowest in those from Northern Ireland.

13. In the case of animals from herds with breeding difficulties, antibodies to Lepto. icterohaemorrhagiae and to Lepto. pomona were detected but antibodies to Lepto. canicola were not found.

Fig. 2 Lepto. canicola, strain 35667 culture viewed by high power, dark ground illumination x1,200.

PART II.

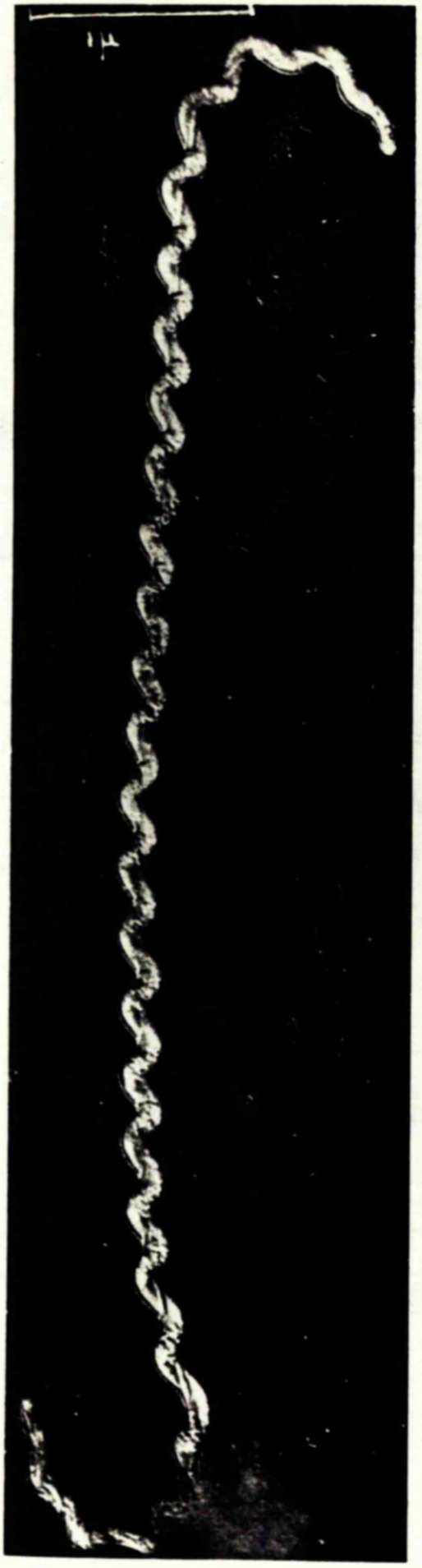
ISOLATION OF Lepto. canicola FROM THE RENAL TISSUES OF NATURALLY INFECTED PIGS

together with

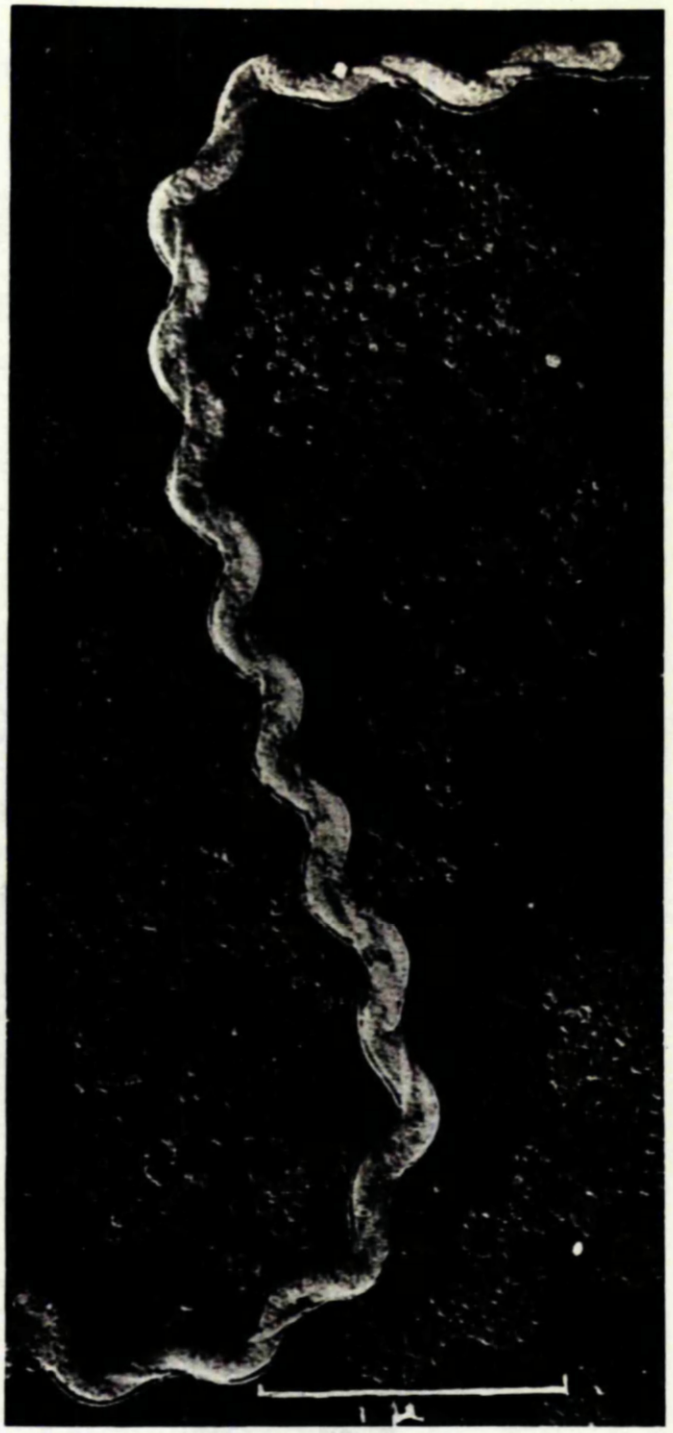
SOME OBSERVATIONS ON THE SURVIVAL OF THE ORGANISM IN TISSUES.

1. INTRODUCTION.

The morphology of Lepto. canicola, as seen by the dark ground microscopy (Fig. 2), does not differ from that of the type species, Lepto. icterohaemorrhagiae. Both are slender, flexuous organisms, 0.1 - 0.15 micron broad and 6 - 12 microns long, though some forms as short as 4 microns and others as long as 25 microns may sometimes be observed (Topley and Wilson's Principles of Bacteriology and Immunity, 4th. Ed. 1955). They demand the same cultural requirements,



×25,000.



×28,000.

Fig. 3. Electron-micrographs of intact *Leptospira canicola*. Freshly fixed, gold-palladium shadowing. (Swain, 1957).

requirements/

but differ in antigenic structure and in their virulence for various animal hosts. Electron microscopy has served to show that both organisms are very similar in morphology and contain a spiral protoplast, 0.1 micron in thickness, which is wound helically around a narrow, straight, axial filament, approximately 0.02 micron in diameter. Both protoplast and axial filament are enclosed within a well-defined cell-wall. The average thickness of an intact spirochaete is 0.14 micron, (Fig. 3: Swain, 1957).

Lukes (1923) probably made the first microscopical observations of Lepto. canicola in stained films of renal tissue and in dark-ground preparations of urine which he obtained from dogs suffering from a clinical condition known at the time as "canine typhus or Stuttgart disease". By means of infective tissue he also transmitted the disease to experimental dogs and named the organism Spirochaeta melanogenes canis (Lukes, 1924).

It was not, however, until 1931 that the organism was isolated at Utrecht, Holland, from the urine of a dog afflicted by a similar clinical entity, which case was subsequently reported by Klarenbeek & Schüffner (1933). When

When/

the antigenic structure of the organism was found to differ from other known species, it was re-named Lepto. canicola (Schüffner, 1934). More detailed investigations by van Riel (1946) led to the recognition of two bio-types of Lepto. canicola, known respectively as the complete, "AB", or "Anvers" strain and the incomplete, "A", or "Roesel" strain. Steigner and Messerschmidt (1950) and Wiesmann (quoted by Wolff, 1954) also pointed out that the antigenic constitution of Lepto. canicola varied to such an extent that, when several strains were tested against a specific antiserum, a diversity of titres was observed. Cross-reactions between Lepto. canicola and the agent of Weil's disease not rarely occur and occasionally may be observed to high titre, especially when the incomplete bio-type of Lepto. icterohaemorrhagiae is involved (Wolff, 1954). Two new types belonging to the Lepto. canicola sero-group have been recently described by Addamiano et al. (1960). Those authors have suggested the name of Lepto. bindjei for the strain obtained from Java in 1938 and the description Lepto. broomi for the strain "Patane", which was isolated by Smith from a human patient in Queensland in 1954.

1954./

Until recently dogs were generally regarded as the only creatures susceptible to infection by Lepto. canicola and as the sole potential carriers of that pathogen (van Thiel, 1948a). Such a belief was later modified by Alston and Broom (1958) after the discovery by Dhont et al. (1934) that the dog was the source of human canicola fever. Later still Lepto. canicola was found to be infective for other animals, notably cattle, jackals and pigs, which in their turn came to act as carrier hosts. In Palestine, van der Hoeden (1955a,b) encountered epidemics as well as sporadic outbreaks of Lepto. canicola infection of cattle and described clinical signs such as fever, loss of appetite, reduced milk-yield, weakness, jaundice and haemoglobinuria. Heavy losses occured among calves and infection appears to have been introduced by jackals. Lepto. canicola was recovered from hedgehogs and also from the ticks that infested them (van der Hœden, 1957). Non-biting flies may also act as vectors of Lepto. canicola (Kumert and Schmidtke, 1952). Serological evidence indicates that horses (Wagener & Mitcherlich, personal communication to Gsell, 1952) and sheep (Mochmann, quoted by Alston and Broom, 1958), too,

too,/

may develop antibodies to Lepto. canicola. There are not a few references in literature to cases of human canicola fever that have been traced to water-borne infection (Walch-Sorgdrager, 1939, Baber & Stuart, 1946, Williams et al. 1953, Warfolomijewa, 1957). Canicola fever of man may also arise by contact with infected pigs (van der Hoeden, 1956, Seiler et al. 1956), or from sources other than dog urine (McIntyre & Seiler, 1953). Especially when they are of mild type, or, as often happens, if they culminate in aseptic meningitis, human infections may be retrospectively disclosed by serological means (Broom, 1951).

2. HISTORICAL.

The original evidence of Lepto. canicola infection of pigs was presented by Williams et al. (1953) who, through the medium of hamsters, isolated the organisms from one out of three samples of pig urine. At the time, the authors were studying in Georgia the epidemiology of canicola fever among 26 human patients who had contracted the disease as a result of bathing in water contaminated by the urine of farm animals. During the same investigation, Ward et al. (1956) obtained Lepto. canicola from the macerated

macerated/

renal tissue of a sow, also *via* hamsters. In Palestine, outbreaks of *Lepto. canicola* infection in two piggeries were described by van der Hoeden (1956) who suspected that infection had been introduced by jackals in the case of, at least, one of the herds. In the absence of clinical illness, the presence of porcine infection came to light in consequence of an outbreak of canicola fever among the piggery workers. Sera from a number of pigs were found to contain *Lepto. canicola* antibodies and the organism was recovered from porcine kidneys. Kmety *et al.* (1956), in a study of leptospirosis in Slovakia, isolated *Lepto. canicola* from the kidneys of three out of 460 healthy slaughtered pigs and, in addition, retrieved four other leptospiral serotypes from 47 of the animals. The incidence of canicola fever in piggery workers in Edinburgh was studied by Coghlan *et al.* (1957) but those investigators failed to recover the organism from the renal tissue of naturally infected pigs, despite the presence in their sera of antibodies to high titre. Warfolomijewa (1957) reported an outbreak among human beings of canicola fever that was contracted from water contaminated by pigs.

pigs./

3. MATERIALS AND METHODS.

In respect of premises on which Lepto. canicola infection had been established by serological means, the kidneys and samples of blood from pigs were collected at the time of slaughter. From three of the infected farms specimens were collected on four different occasions. Usually, the materials were delivered to the Department within 1 - 5 hours of death. The initial three kidneys of the series, together with the corresponding blood samples, were obtained in February 1957 and represented a group of five animals sent for slaughter from a farm near Muirhead, in the county of Dunbarton. Renal tissue was extracted through the seared capsule by means of Pasteur pipette and was seeded into Schüffner's medium, modified by Stuart (1946), which was contained in amounts of 3.5 ml. in screw-capped bottles of miniature, or bijou, size. Once the pipette had been plunged into the kidney, it was not withdrawn until, at least, six cores of tissue had been recovered and all the material so obtained was transferred to one bottle of medium. For each kidney four bottles of medium were used. Cultures were then incubated aerobically at 32°C and were examined at fixed weekly intervals by means of dark-ground illumination under a 16 mm.

16 mm./

objective in combination with an x20 ocular. All cultures were maintained for five weeks after which negative ones were discarded.

For biological examination, approximately, 2 grammes of material from diverse parts of each kidney were ground up in a mortar with sterile silver sand and suspended in normal saline solution. So that the sandy particles might settle, the suspension was set aside for five minutes and was then centrifuged at 1000 rpm. for three minutes so that gross fragments of tissue might deposit without, it was hoped, simultaneous removal of too many leptospirae. One millilitre of the supernatant fluid was inoculated intraperitoneally into a guinea-pig, 800 grammes in weight.

The residual supernatant fluid was examined microscopically by dark-ground illumination under a 2 mm. objective and an x10 ocular and similar examination was made of urine recovered from the renal pelvis. For histopathological examination, pieces of kidney were fixed in 10 per cent. neutral formol-saline solution.

Samples of blood were left overnight in room temperature ere the sera were recovered and tested with suspension of Lepto. canicola, Lepto. icterohaemorrhagiae, Lepto.

Lepto./

grippotyphosa, Lepto. hyos and Lepto. pomona for agglutination-lysis by the technique of Schüffner, as described by Wolff (1954). On the fourth day after inoculation heart's blood, procured by cardiac puncture, and peritoneal fluid were taken from the guinea-pigs and sown into Schüffner's medium, six bottles of which were used for each sample of material, amounting to twelve bottles in all. The cultures were then incubated and examined in the way already described.

In October 1957, material for the isolation of Lepto. canicola was forthcoming from 14 animals that had been reared on premises at Bearsden, just outside Glasgow, where some weeks before one of the attendants had developed an illness that was diagnosed as canicola fever. At that time Dr. J. C. J. Ives, of Glasgow Royal Infirmary, found a high titre of antibodies to Lepto. canicola in the sera of several in-contact pigs as well as in the serum of the worker. Of three dogs on the farm, two reacted serologically to both Lepto. canicola and Lepto. icterohaemorrhagiae but the third animal showed evidence of infection only by the latter organism. From each of 14 pigs, out of a batch of 25, sent to a bacon factory for slaughter, a sample of blood of about

about/

25 ml. in amount was obtained during the course of bleeding-out and one kidney was procured as soon as the carcass had been eviscerated. The laboratory procedures followed in this case were similar to those just described save that, for biological examination, hamsters replaced guinea-pigs. To economise in experimental animals, the suspensions of renal tissue were pooled into five lots, 2 ml. of each of which were inoculated intraperitoneally into Syrian golden hamsters (Mesocricetus auratus) of, approximately, six weeks of age. Five days after inoculation, from each hamster heart's blood and peritoneal fluid were sown, in quantities of two drops and one drop, respectively, into two groups of four miniature bottles of modified Schüffner's medium that were subsequently incubated and examined as already described for the pig kidneys.

Towards the end of April 1958 material for the isolation of Lepto. canicola was obtained on two occasions from a piggery at Condorrat, 12 miles outside Glasgow. About that time the owner had dispatched 30 pigs for slaughter at a bacon factory. As on previous occasions, a blood sample and one kidney were procured from each of 15 of the animals and,

Fig. 4. Minute whitish foci and petechial haemorrhages in the pig kidney from which Lepto. canicola (strain 35667) was recovered.

and,/

within five hours of decease, the kidney material had been seeded into Schüffner's medium. The technique used in this case was identical with that followed in previous instances. Two months later, on a visit to the same farm, I obtained a stunted pig which was one of a group of 13, of which all the other members were in good health and almost ready to be slaughtered for human consumption. Autopsy, conducted within an hour after slaughter of the animal, revealed a diaphragmatic hernia in which a length of about one foot of the small intestine projected into the thoracic cavity and which was associated with fibrinous inflammation of the adjacent peritoneum. Fibrino-haemorrhagic colitis was also present. The cortex and the medulla of both kidneys contained numerous yellowish foci, most of which measured, less than 1 mm. in diameter, but a few were of slightly larger size (Fig. 4). The serum of the creature was subjected to serological examination and cultures were made in the usual way from both kidneys.

The kidneys of the Condorrat pig were further used for a study of the survival of leptospirae in renal tissue. For a total of 13 days, the renal material was stored in a sterile glass container in a refrigerator, at a temperature of 0° to

to/

$4^{o}C$. Day by day, ten to twelve bijou bottles were inoculated with fragments of kidney tissue whilst other portions, of approximately 2 grammes in weight, were macerated and films made from the supernatant fluid after centrifugation and examined by dark-ground microscopy. The centrifuge tubes, containing both macerated material and supernatant fluid, were then set aside at room temperature and similarly examined every day for the next six days. In two instances, the reaction of the newly macerated renal tissue was estimated in the Department of Veterinary Physiology by means of an E.I.L. Vibron electrometer. In all other cases, the pH. values were obtained by means of a B.D.H. capillator. Similar studies were later made on the survival of Lepto. canicola (a) after that organism had been inoculated into the renal tissue of a pig that did not posess any leptospiral antibodies, and (b) in the kidneys of a naturally infected dog that showed a titre of specific antibodies of 1:100,000. Additonally, samples of dog and of pig urine were observed for loss of motility and time of complete disintegration of Lepto. canicola at different levels of hydrogen - ion concentration. For that purpose, undiluted urine, free from leptospiral antibodies, was distributed in 2 ml. amounts into sterile bijou bottles. The addition of

of/

measured amounts of sterile lactic acid or of sterile sodium hydroxide, in 1:100 and 1:1000 dilutions, respectively, provided a series of specimens, the reactions of which ranged from pH = 5.2 to pH = 8.0. Each of the bottles was then inoculated with 0.25 ml. of a suspension of Lepto. canicola, Aldgate strain, and after they had been thoroughly shaken, were left at room temperature. At different intervals thereafter, films were examined by means of dark-ground microscopy under low power magnification as well as under an oil-immersion objective.

4. RESULTS.

From two out of three kidneys received from Muirhead in February 1957, leptospirae were grown in Schüffner's medium. The first culture (No. 860) came from the kidney of an animal in which Lepto. canicola antibodies occured to a dilution of 1:3000 of its serum and growth was observable within 14 days of inoculation. Out of four bottles of media inoculated, three yielded leptospirae. The other culture (No. 861) was recovered from the kidney of an animal which was found to have a serum titre of Lepto. canicola of 1:300. In this case growth was noticeable in but one out of four culture bottles and then only after five weeks of inoculation. From the third animal, which had a serum-titre of 1:100, cultures

cultures/

were not obtained.

After they had become well accustomed to artificial conditions of growth, cultures Nos. 860 and 861 were first subjected to agglutination-lysis tests against five standard rabbit leptospiral antisera and also against the pig serum that showed the highest titre. Both cultures were found to give a positive reaction with Lepto. canicola rabbit antiserum to a dilution of 1:10,000, but reacted with the pig serum to a dilution of only 1:3000. Both cultures, too, gave cross-reactions with Lepto. icterohaemorrhagiae antiserum to a dilution of 1:100. Tests with Lepto. grippotyphosa, Lepto. hyos and Lepto. pomona rabbit antisera proved negative. Thus a tentative diagnosis of infection by Lepto. canicola was made, which opinion was subsequently confirmed by the late Dr. J. C. Broom after he had carried out absorption tests on one of the cultures (No. 861). Attempts to isolate the organism by inoculation of macerated kidney into guinea-pigs were unsuccessful and an appreciable titre of antibodies in the sera of those animals was not demonstrable two weeks after injection. Dark-ground microscopical examination of the supernatant fluid from macerated kidneys was inconclusive in one case (No. 860) and negative in the other two. In drops of urine

urine/

recovered from the renal pelvis leptospirae were not found by microscopical means. In this instance, therefore, direct cultivation from renal tissue proved the most satisfactory method of detection of leptospira.

It was consequently hoped that leptospirae might be similarly isolated from the second set of specimens, which consisted of the kidneys of 14 animals and were accompanied by corresponding samples of blood. Although the pigs had been reared under similar environmental conditions and were of the same killing and of similar age groups, the titres of serum-antibody proved far from uniform, as indicated in Table 13.

TABLE 13.

TITRES OF LEPTOSPIRAL ANTIBODIES IN SERA OF PIGS FROM BEARSDEN, GLASGOW.

	Leptospira antigen:				
Pig serum samples:	canicola	ictero-h.	grippo-t.	hyos	pomona
1067	1:3,000	1:100	-	-	-
1068	1:1,000	1:30	-	-	-
1069	1:30,000	1:100	-	-	-
1070	1:100	1:10	-	-	-
1071	1:30	-	-	-	-
1072	1:100	1:10	-	-	-
1073	1:10,000	1:300	-	-	-
1074	1:300	1:10	-	-	-
1075	1:10,000	1:100	-	-	-
1076	1:3,000	1:10	-	-	-
1077	1:100	-	-	-	-
1078	1:1,000	1:30	-	-	-
1079	1:30	-	-	-	-
1080	1:1,000	1:30	-	-	-

Even at the lowest dilution employed, i.e. 1:10, not one sample reacted with either Lepto. grippotyphosa or Lepto. hyos or Lepto. pomona, whereas with Lepto. canicola all the sera manifested titres that varied from 1:30,000. Cross-reaction occurred with Lepto. icterohaemorrhagiae save with three samples (Nos. 1071, 1077 and 1079). Lepto. canicola (strain 1078), (Michna, 1959a) was recovered from only one kidney. The strain proved rather slow to develop under artificial conditions and, indeed, its growth in culture was not observable until the fifth week of inoculation. In the remaining cultures heavy contamination by organisms of the coliform type occurred and may have been responsible for the failure of leptospiral growth. Isolation of the organism via hamsters also proved unsuccessful and microscopical examination of films of macerated kidneys, too, failed to reveal the presence of leptospirae.

The reactions of strain No. 1078 with the five standard rabbit leptospiral antisera and with pig serum 1078 are recorded in Table 14.

TABLE 14.

AGGLUTINATION-LYSIS TEST OF LEPTO. CANICOLA, STRAIN 1078, WITH RABBIT LEPTOSPIRAL ANTISERA AND WITH PIG SERUM, 1078.

Antisera:	Titres:
Lepto. canicola	1:30,000
Lepto. ictero-h.	1:1000
Lepto. grippo-t.	-
Lepto. hyos	-
Lepto. pomona	-
Pig serum 1078	1:3000

That the culture was in fact one of Lepto. canicola was subsequently confirmed by the late Dr. J. C. Broom.

The virulence of strain No. 1078 was tested in a guinea-pig of 530 grams of weight and in a hamster six weeks old, both of which animals were inoculated intraperitoneally with 2 ml. and 1 ml., respectively, of a third generation subculture in Schiffner's medium. One hour after inoculation a sample of blood from each animal, secured by cardiac

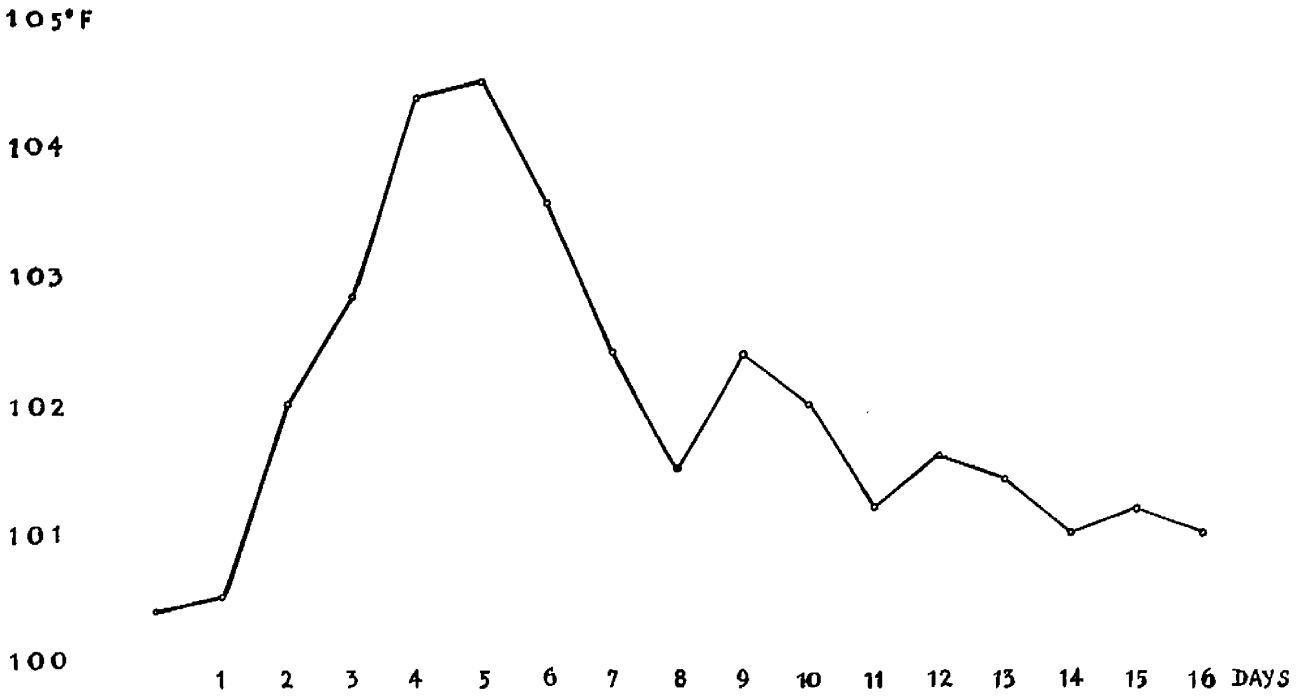


Fig. 5. - Temperature chart of guinea-pig inoculated with Lepto. canicola, strain 1078.

cardiac/

puncture, was sown into three bottles of Schüffner's medium which were incubated at 32°C. In cultures from the hamster, leptospirae were demonstrable as early as the third day of incubation, whereas in those from the guinea-pig the organisms were not observable before the sixth day. In the case of the guinea-pig, further cultures were made on the third day after inoculation and were found to yield a good growth of leptospirae by the tenth day of incubation. Apart from transient loss of appetite, the only clinical response was a thermal reaction which is illustrated in Fig. 5, whilst, two to three weeks after infection its serum was found to contain Lepto. canicola antibodies in a dilution of only 1:30. The hamster was found dead on the morning of the sixth day after inoculation and cultures from the liver and kidneys yielded organisms, mainly of the coliform type.

In the case of a third group of kidneys, received from Condorrat, more success attended both the microscopical demonstration and the isolation of leptospirae. Out of fifteen kidneys three were characterised by petechial haemorrhages which occurred throughout the entire organ. Four of the related samples of sera proved negative to agglutination-lysis; one gave a titre of 1:100 to Lepto. icterohaemorrhagiae

icterohaemorrhagiae/

only, whilst the remaining ten samples contained antibodies to Lepto. canicola in dilutions ranging from 1:300 to 1:30,000. The 15 kidneys were macerated and pooled into five lots, in two of which leptospirae were demonstrated by microscopical means. One millilitre of each lot of macerated renal tissue was then injected intraperitoneally into a hamster and leptospirae were subsequently recovered from the liver and the kidneys of three of them. The remaining two hamsters were found dead 30 hours after injection and Past. septica was procured from the internal organs. Six out of the 15 kidneys sown into Schüffner's medium yielded cultures of leptospirae (strains Nos. 2046, 2049, 2052, 2053, 2054 and 2055). Cultures 2046 and 2052 gave an abundant growth that was observable within one week of incubation but, in the case of the other four strains, organisms were detectable microscopically only after incubation for two to three weeks. Nine kidneys gave a profuse growth of Past. septica.

The fourth generation of the six strains of isolated leptospira was injected intraperitoneally into golden hamsters in 1 ml. amounts and all of the experimental animals died five to eight days after infection. All six

six/

strains were subjected to serological examination in which the usual five standard rabbit leptospiral antisera were used, and were found to react positively only with Lepto. canicola and Lepto. icterohaemorrhagiae antisera to titres which varied from 1:3000 to 1:30,000 and from 1:30 to 1:100, respectively. A presumptive determination of Lepto. canicola was subsequently confirmed by the late Dr. J.C. Broom.

During June 1958, three visits were made to the Condorrat farm not only to obtain information concerning husbandry, breeding and environmental conditions but also to attempt to trace the origin of such an extensive infection of pigs by Lepto. canicola. At the first visit, the owner stated that some years before two pigs died, apparently from jaundice, but the etiology was not investigated. At the time, the piggery was a self-contained one, and breeding difficulties were not reported although a piglet occasionally died soon after birth, ostensibly as a result of "crushing", and still-born piglets were sometimes encountered. The animals were fed mainly on swill, supplemented by a manufactured diet which did not contain antibiotics. Because of a poor breeding record one sow had been discarded. Of three unthrifty piglets, one was stunted and suffered from chronic diarrhoea whilst the

the/

other two had a temperature of 103°F. and manifested patches of erythema over the abdomen as well as on the ears and the inside the thighs. There were some rats on the premises and a few years previously the owner kept a dog for the purpose of catching those vermin. At the time of visit, the only other creature on the premises was a pet French poodle which appeared to be in good health, and there was not any history of illness among the piggery-workers. The urine and all fluids from the piggery floors drained into an underground pipe which, at a point about 70 yeards away from the buildings, discharged into an open stream, that flowed into a pond, three to four hundred yards distant. A few mixed samples of urine were taken from several of the pigs and fluid was also collected from the floors of five of the pens. About 200 ml. of the effluent were collected at the outlet of the main drain. Microscopical examination of the pooled urine samples failed to reveal the presence of leptospira, but those organisms were found in the samples of fluid collected from the floors of four out of the five pens. Numerous, actively motile leptospirae were present in films made from the effluent of the drain.

A second visit was paid four days later when blood samples were collected from several animals, including

including/

two breeding sows. Only one of the samples, which had been taken from the stunted pig already mentioned, reacted to Lepto. canicola to a dilution of 1:30,000 and Lepto. icterohaemorrhagiae to 1:1000. To both of those antigens, the sera of the two sows had a titre of 1:100.

The farm was again visited on June 25 by which time the red patches on the skin of the two piglets previously described had disappeared, the body temperature had dropped to 102.4°F. and other clinical signs were not manifest. The stunted pig was acquired and its kidneys were later used for the isolation of Lepto. canicola (strain No. 35667), the serological identity of which is attested in Table 15.

TABLE 15.

AGGLUTINATION-LYSIS TESTS OF LEPTO. CANICOLA, STRAIN No. 35667.

Antigen:	Rabbit leptospiral antisera:					Pig serum No. 35667.
	canicola	ictero-h.	grippo-t.	hyos	pomona	
Lepto. canicola No. 35667	1:10,000	1:100	-	-	-	1:30,000

The isolated culture proved fatal to a ten-weeks-old golden hamster, which animal died 12 days after intraperitoneal inoculation of 1 ml. of a third generation subculture. A guinea-pig survived similar inoculation of a dose of 2 ml. although, several days after injection, it manifested a thermal reaction and about 15 days later its serum was found to contain Lepto. canicola antibodies to a dilution of 1:100.

The late Dr. J.C. Broom, who carried out absorption tests of the strain, reported: "Our recent findings are that the Lepto. canicola, Utrecht antiserum with an unabsorbed titre of 1:10,000 was reduced to 1:3000 after absorption with strain 35667. The 35667 also had an homologous titre of 1:10,000, which after absorption with the Utrecht strain was reduced to 1:300. These findings are very similar to the ones we had with strain 1078". It would appear, therefore, that the two cultures, Nos. 1078 and 35667, were antigenically similar although they were recovered from the renal tissue of pigs reared on different farms. Moreover, the two strains were not wholly identical with the standard Utrecht strain of Lepto. canicola. For the present, it is an open question whether cultures Nos. 1078 and 35667 are varieties of the standard Utrecht strain or are incomplete serotypes of Lepto. canicola.

5. OBSERVATIONS ON THE SURVIVAL OF Lepto. canicola.

(a) Persistence in chilled and frozen porcine kidney.

In an earlier paper (Michna, 1959a) it was suggested that the kidneys of carrier pigs may be the source of infection of cases of human canicola fever of unknown origin and that the consumption of those organs in the raw state may be responsible for leptospirosis of dogs and cats.

A plentiful supply of suitable material afforded an opportunity to carry out studies on the survival of Lepto. canicola in porcine renal tissue and some results of that investigation have been published (Michna, 1959b). Observations were limited to, mainly, loss of motility on the part of the organism and its relation to leptospiral survival in tissues that were maintained at different temperatures of storage.

Table 16 shows that motile leptospirae were demonstrable for seven days in kidney tissue that was stored at 0-4°C. Non-motile forms were observable for two further days. In a saline suspension of kidney tissue maintained at room temperature, 18°C., leptospirae were found to remain motile for only one to three days.

It was noted, too, that the onset of bacterial contamination coincided with fairly rapid loss of motility

TABLE 16.

MICROSCOPICAL EXAMINATION OF MACERATED PIG KIDNEYS.

Macerated on:	Examined on:											
	25/6	26/6	27/6	28/6	29/6	30/6	1/7	2/7	3/7	4/7	5/7	6/7
25/6	+	+	+	+	c. n.m.	-	-					
26/6		+	+	+	c. n.m.	-	-					
27/6			+	+	+	c. n.m.	-	-				
28/6				+	+	+	c. n.m.	-	-			
29/6					+	c. n.m.	-	-				
30/6						+	+	+	c.	-		
1/7							+	+	c.	-		
2/7								+	c.	-		
3/7									n.m.	-	-	
4/7										n.m.	-	-
5/7											-	-

+ = motile leptospira c. = contaminated.

n.m. = non-motile leptospira - = negative.

motility/

followed by disappearance of the leptospirae. Concurrently, the acidity of the medium increased to pH = 5.9, or more, as shown in Table 17.

TABLE 17.

PRESUMPTIVE EFFECT OF HYDROGEN-ION CONCENTRATION ON LEPTOSPIRAE.

Date of maceration:	Date of estimation:	pH.	Microscopical findings
25/6	29/6	5.9	Non-motile, (Contaminated).
26/6	30/6	5.8	Negative. (Contaminated).
27/6	27/6	6.735	Many motile leptospirae.
27/6	30/6	5.9	Non-motile, (Contaminated).
28/6	30/6	6.1	Motile leptospirae.
29/6	30/6	5.9	Non-motile, (Contaminated).
30/6	30/6	6.735	Many motile leptospirae.

The critical level of acidity appears to be about pH = 6.0 by which reaction the motility of leptospirae is not affected but below which the organisms speedily become sluggish

sluggish/

and clump together. Should bacterial contamination occur, however, the leptospirae disappear within 24 to 48 hours.

TABLE 18.

CULTIVATION OF LEPTO. CANICOLA FROM PIG KIDNEYS CHILLED AT 0-4°C.

Media inoculated on:													
25/6	26/6	27/6	28/6	29/6	30/6	1/7	2/7	3/7	4/7	5/7	6/7	7/7	8/7
*11/12	5/10	6/10	5/10	10/12	12/12	7/10	7/10	9/10	6/10	-	2/10	1/10	-
			*Growth in:11 bottles out of 12 inoculated.										

Leptospirae were recovered from pig kidneys, chilled at 0-4°C. for up to 12 days after slaughter. In Table 18 the numerator denotes the number of cultures in which leptospiral growth occurred and the denominator indicates the total number of cultures procurable on a given day. Thus, 11 out of the 12 cultures, made on the day on which the animal died, proved positive and in some of these cultures growth was observed as early as the fifth day of incubation. Whenever bacterial contamination of a culture interposed, however, leptospirae were not found. By the eleventh to the twelfth day of storage, the vitality of Lepto. canicola in renal tissue had markedly declined inasmuch as only two and one bottles, out of two groups of ten originally inoculated, were found to contain satisfactory growth, although the residual cultures remained free from bacterial contamination. Since the cultures prepared on the 13th day of storage proved negative for leptospira after five weeks of incubation, the organism was assumed not to have survived for that length of time under the particular experimental conditions.

The survival of Lepto. canicola was also investigated after the kidneys, procured from an animal free from infection, had been inoculated with a culture of the Aldgate strain. The object of that experiment was to determine the

the/

extent to which contamination of sound carcasses might be caused by the handling of, or contact with, the body of a carrier animal. One millilitre of culture was introduced into each of the two kidneys, which latter were than placed in a refrigerator where they were maintained at a temperature of 0-4°C. Two days later, one of the kidneys was transferred to a low temperature cabinet where it was exposed to -8°C. In the case of the kidney stored at 0-4°C. motile leptospirae were observable for up to eight days and the organism was recovered *via* Schüffner's medium for periods of up to 21 days. In the instance of the kidney frozen at -8°C. leptospiral motility ceased after three days and cultures were obtainable for six days, after which time that mode of examination was discontinued because of pressure of other work.

(b) Persistence in chilled canine kidney.

In the case of naturally infected dog, having serum titres of 1:100,000 and 1:10,000 to *Lepto. canicola* and *Lepto. icterohaemorrhagiae*, respectively, *Lepto. canicola* was found to survive in renal tissue for a relatively short time. Organisms were observable for up to seven days in films made from macerated kidneys and were cultivated for nine days after storage at 0-4°C.

It is here noteworthy that the urine of the creature was found to contain numerous motile leptospirae for up to three days during which time the reaction remained at pH = 6.8 - 7.4. On the fourth day, when the reaction of the urine reached pH = 7.8 only non-motile organisms were demonstrable.

(c) Persistence in urine.

Microscopical examination for motility and observations concerning the disappearance of leptospiral forms in artificially infected, undiluted samples of pig urine of different pH concentration have yielded interesting results. Thus, in fluids, of pH = 5.2 - 5.6, the organisms lost their motility within one hour and at pH = 5.2 organisms had disappeared after two days. In fluids of pH = 5.4 - 5.6 the gross morphological features were appreciable for four and for five days, respectively. At pH = 5.8, leptospiral motility ceased after 24 hours, but the organisms were demonstrable by microscopical means for six days. In fluids of pH = 6.0 - 7.4, motility remained very active until the fifth day, declined on the sixth day and was lost after seven days. In those samples that escaped bacterial contamination, some non-motile leptospirae were visible in films made eight days after observations began but organisms were not to be found

TABLE 19.

MICROSCOPICAL EXAMINATION FOR MOTILITY AND DISAPPEARANCE OF LEPTO. CANICOLA IN ARTIFICIALLY INFECTED, UNDILUTED PIG URINE OF DIFFERENT pH.

found/

by the eleventh day. At maximal alkalinity (ph = 8.0) motility was markedly reduced after 24 hours and had ceased completely by the fourth day. In fluids of pH = 7.8 - 8.0, organisms were not observable by the fifth day. Detailed results of microscopical examination for the presence of motility and disappearence of leptospira in artificially infected, undiluted samples of pig urine are presented in Table 19.

6. DISCUSSION.

The isolation of leptospira from the internal organs of the body or from urine provides essential proof of infection but laboratory cultivation may be severely influenced by altogether too many factors. Serological evidence of the existence of Lepto. canicola infection of pigs on a farm at Muirhead was subsequently confirmed by isolation of the organism from two out of three kidneys, (culture Nos. 860 and 861) and the serum-titres of antibody in the two pigs were 1:3,000 and 1:300, respectively. Growth was not obtained from the kidney of an animal with a serum-titre of 1:100. The latter titre may either indicate early infection or be attributable to residual antibodies from past infection. In the former instance recovery of the organism from viscera may be

be/

confidentially expected, whereas failure to cultivate may rather denote bygone infection. It was disappointing, therefore, that only one strain (No. 1078) was forthcoming from a group of 14 kidneys, especially, because organs had been procured from animals which manifested serum-titres ranging from 1:30 -- 1:30,000. It is further noteworthy that strain No. 1078 came from a pig with a serum-titre of 1:1000 and that similar, or higher titres were recorded from six other animals in the group. Since most of the other 13 cultures were found to contain gram-negative organisms of the coliform type the low rate of success attending artificial cultivation was ascribed to bacterial contamination. Whether the latter occurred ante-mortem or took place at a later stage it was impossible to establish. Despite a concurrent infection by Past. septica, more satisfactory results were obtained in the case of kidneys from Condorrat. Seven cultures of Lepto. canicola (strain Nos. 2046, 2049, 2052, 2053, 2054, 2055 and 35667) were grown from 16 kidneys. The presence of Past. septica, however, was not without its effect on attempts to recover leptospira via hamsters since two out of five of the rodents died of pasteurellosis within 30 hours. Culture Nos. 2046 and 35667 were procured from animals with a serum-titre of

of/

1:30,000 and cultures 2049, 2052 and 2054 came from pigs with a serum-titre of 1:10,000. Cultures Nos. 2055 and 2053 were isolated from animals with serum-titres of only 1:1000 and 1:300, respectively. Although the piggery was known to be heavily contaminated by Lepto. canicola, the sera of five pigs of the batch did not contain any antibodies. Out of 15 animals from the same piggery, that were consigned to a bacon factory, three had kidneys that manifested small haemorrhages but, none the less, their carcasses were passed for human consumption.

As illustrated by Fig. 4, both kidneys from the stunted pig exhibited small whitish foci together with minute haemorrhages, which lesions might well have been overlooked during routine meat inspection. It is not impossible, therefore, that pig carcasses infected by Lepto. canicola may be passed for human consumption. It may here be recalled that renal tissue has been shown to remain infective for at least twelve days at 0-4°C. and for not less than six days at -8°C. Should a pig be slaughtered during the stage of leptospiraemia, the organisms are likely to be found in the blood and in internal organs, such as the liver, spleen, lungs and the carcass. The handling of such material, therefore, may be

be/

hazardous to man and, indeed, human infection of that origin has been reported by Bernkopf (1948) in respect of Lepto. grippotyphosa and by Johnson (1950) with regard to Lepto. pomona.

It may be of some interest, to laboratory workers especially, that O'Connel and Broom (1952) were able to isolate Lepto. icterohaemorrhagiae from rat kidneys that had been kept for up to three days at 5°C. The voided urine of carrier animals may constitute another source of infection by Lepto. canicola since that organism has been shown to remain alive for about a week in the case of undiluted and uncontaminated urine and for a much longer period in urine diluted with rain water. In passing, it may be noted that Kirschner & Maguire (1957) found that Lepto. pomona could survive for up to two months in cow urine that had been diluted to 1:10 - 1:100 with tap water, a finding that may be of significance to public health.

7. SUMMARY.

1. Ten cultures of Lepto. canicola have been obtained from the renal tissues of naturally infected pigs, reared on three different farms in the vicinity of Glasgow.

Glasgow./

2. The presence of infection on those premises was previously detected by serological means.

3. Culture No. 1078 was recovered soon after canicola fever had been diagnosed in a worker employed on the farm.

4. In a study of the survival in pig kidney of Lepto. canicola (strain No. 35667) it emerged that the organism could survive for twelve days in naturally infected material stored at 0-4°C., and for 21 days in artificially infected normal kidney maintained under the same conditions.

5. When, after it had been kept for two days at 0-4°C., artificially infected kidney was transferred to a storage temperature of -8°C., Lepto. canicola was recoverable for up to six days.

6. In naturally infected canine kidneys preserved at 0-4°C., Lepto. canicola (culture No. 36482) survived for up to nine days.

7. The persistence of Lepto. canicola in artificially infected urine kept at room temperature (18°C.) appears to be favoured by a level of pH = 6.2 - 7.4, provided that bacterial contamination does not ensue. Under such conditions leptospirae may be detectable for up to seven days.

days./

8. At pH = 5.2 - 5.6, leptospirae in urine may lose their motility within one hour and may disappear altogether within two to five days.

9. Should urine become contaminated by bacteria, loss of motility and subsequent disappearance of the leptospirae are greatly accelerated.

PART III.

STUDIES ON THE DEVELOPMENT OF ANTIBODIES IN THE SERUM OF PIGLETS EXPERIMENTALLY INFECTED BY Lepto. canicola.

1. HISTORICAL.

The first record of the use of piglets in experiments on leptospirosis was that of Uhlenhuth and Fromme (1930) who found that injection of a culture of Lepto. icterohaemorrhagiae into one animal was without any effect. Their consequent claim that the pig was naturally resistant to infection by the agent of Weil's disease was supported by Melanidi et al. (1933). Klarenbeek and Winsser (1937) isolated the organism from an ailing piglet and so produced evidence that pigs were susceptible to natural infection by Lepto. icterohaemorrhagiae. The same authors, however, found the only response in two experimental piglets to be the presence of specific antibodies in sera to dilutions of 1:10 and 1:30, respectively. Schmid and Giovanella (1947), Burnstein and Baker (1954) and Bohl et al. (1955)

(1955)/

succeeded in infecting piglets with Lepto. pomona. During a study of abortions and neonatal mortality in pigs, Tammemagi and Simmons (1958) infected five sows with Lepto. hyos (mitis). In the available literature concerning experimental infection of piglets by Lepto. canicola, the only report appears to be that of Coghlan et al. (1957). By various routes, six piglets aged from 2 - 3 months, were infected by means of a culture of Lepto. canicola, Aldgate strain. The animals remained clinically normal and in four of them the only sign of infection was the appearance of specific antibodies in the serum. Unsuccessful were attempts to demonstrate the presence of leptospirae in the blood and urine and to isolate the organism from the internal organs, either culturally or via hamsters. In another experiment, two piglets were infected, one by subcutaneous inoculation of 3 ml. of pig urine containing many leptospirae and the other by application to the scarified skin of cotton-wool which had been soaked in that secretion. Pyrexia was the only clinical finding and was followed by the development of specific antibodies in the serum. Lepto. canicola was recovered from the renal tissue of one of the pigs. The authors concluded that pigs may harbour Lepto. canicola and so constitute healthy

healthy/

carriers, the urine of which is a potential source of infection for other animals and for man.

The experimental work now under report was designed to study the antibody response of piglets infected by Lepto. canicola. At the same time, the isolation of leptospirae from blood and from the internal organs was carried out at different stages of infection. Additional data relating to clinical signs, such as body temperature, appetite and diarrhoea, and to post-mortem findings as well as to haematological changes were also collected. Samples of urine were subjected to microscopical examination for evidence of leptospiruria. Finally, studies were continued on the capacity of Lepto. canicola to survive in pig renal tissue maintained at 0-4°C.

2. MATERIALS AND METHODS.

Lepto. canicola, strain No. 35667 recently isolated from pig renal tissue (Michna 1959b), was used throughout this part of the work. The organism was grown in Stuart's modification of Schiffner's medium. Profusely growing and actively motile cultures provided the infective material. The experiments were carried out during the period, August 1959 to June 1960, and involved three successive groups of animals. Two

Two/

experiments each involved seven piglets which were divided into two groups of three infected animals whilst the remaining creature acted as a control. In the third experiment, 11 piglets were infected and were kept in lots of 4, 4, and 3 and, again, the twelfth creature was kept as a control. Thus, the investigation extended to a total of 26 animals, all of which were born of sows that did not contain any leptospiral antibodies in their sera. After weaning, the experimental stock was fed on dry 'B.O.C.M.' creep mash for two weeks before they were gradually transferred to weaners food, without any antibiotic supplement. Water was supplied ad libitum. At the time of delivery, all animals were lively and in excellent bodily condition. When studies were begun, piglets of the first group, Nos. 1-7, were eight weeks of age; those in the second experiment, Nos. 8-14, were six weeks old and the remaining, Nos. 15-26, were five weeks of age, with the exception of piglets 18 and 19, which had been born only three weeks earlier.

The serum of all the experimental animals was subjected to the agglutination-lysis test in which all the types of leptospirae involved in Part I of this work were employed. Body temperatures were taken and blood samples

samples/

for haematological examination were secured. The piglets were kept in adjacent pens separated by a cement partition-wall, two inches thick and three feet high. The cement floors were not only smoothly finished and slightly sloped but were also provided with rounded corners whereby accumulation of urine and faeces was minimised and cleansing and disinfection of the pens, done every second day, was more thoroughly effected. To obviate the possibility of re-infection from urine on the floor, an adequate supply of saw-dust and hay for bedding was provided. Strict precautions were taken to ensure that infection would not be spread to other parts of the animal house. The technical personnel were required to wear protective rubber gloves, boots and aprons. Both outside and inside the door of the room were placed metal trays, each several inches deep and filled with 5 per cent lysol, through which a person passed when entering and leaving the cubicle. Sacs soaked in disinfectant were spread over the gangway, and all the cleansing fluids were made to pass through a filter, also containing disinfectant, before they entered into the drains.

In the first series of experiments, piglets Nos. 1, 2 and 3 received subcutaneously, into the left thigh, 1 ml. of

of/

a culture of Lepto. canicola, strain No. 35667. Piglets Nos. 4, 5 and 6 were infected by scarification of the skin behind an ear by means of an hypodermic needle that was previously dipped in culture so that they acquired a considerably smaller amount of the infective dose. Piglet No. 7 was not infected and served as a control animal.

In the second train of experiments, scarification of the skin as already described was the mode of infection of all animals save Piglet No. 14 which remained as a control.

In the final part of the experimental work piglets Nos. 15 - 25 were given, by the subcutaneous route 0.5 ml. of a culture of Lepto. canicola and piglet No. 26 was retained as a control.

The animals were examined daily and rectal temperatures were recorded, as shown in the temperature charts (Figs. 7, 13, 20, 25, 32 and 36).

During the four weeks of the initial experiment, blood samples were collected by venipuncture from the jugular vein at weekly intervals for purposes of cultural, serological and haematological examinations. One month after infection blood samples were taken at bi-weekly intervals for haematological and serological examinations only. Piglets in each

each/

group were killed at different times after infection, which varied from two weeks to nearly four months. A fortnight after infection one animal from each of the infected group (piglets Nos. 2 and 5) was killed, the internal organs were examined macroscopically and cultures made from the heart's blood, kidneys, liver, spleen, lungs, mesentoric lymph-nodes and, in the case of piglet No. 2, from the gluteal muscles of the inoculated leg. For each organ or tissue, ten bijou bottles, each containing 2.5 ml. of medium, were used. The kidneys were macerated, suspended in sterile saline solution, centrifuged for 3 minutes at 1000 rpm and films made from the supernatant fluid for examination by dark-ground microscopy. After the second week of infection samples of urine were checked at weekly intervals for leptospiruria.

Piglets Nos. 3 and 6 were killed three months after the experiment began. The remaining three animals (piglets Nos. 1, 4 and 7) were sacrificed after a period of 15 weeks.

During the second and third experiments blood cultures for leptospirae were made four days after infection. In the case of the second group of animals blood culture was continued at weekly intervals for a month, by which time it became clear that leptospiraemia lasted for a week at most so

so/

that blood culture was performed at only one week after infection in the case of the third experiment. For serological and haematological examinations blood samples from the second group were procured at weekly intervals for a month and thereafter every two weeks. Samples from the piglets of the third group were collected one week after infection and afterwards at bi-weekly intervals.

Piglets of the second group were killed as follows: Nos. 10 and 12 after four weeks and Nos. 8, 9, 11, 13 and 14 at the end of seven weeks after the start of the experiment.

In the case of the third group, after infection, piglet No. 15 was killed at three-and-a-half weeks; piglets Nos. 21 and 22 at six weeks; piglets Nos. 23 and 24 after eight weeks; piglets Nos. 17, 19 and 20 after ten weeks and piglets Nos. 16, 25 and 26 at the end of three months from the beginning of the experiment.

The laboratory procedures followed were essentially the same as in the previous experiments but, in some cases, fewer bottles of culture-media came to be used. All samples of serum were subjected to agglutination-lysis tests in which Lepto. canicola, homologous No. 35667 strain, stock Aldgate culture

culture/

of the same organism, Lepto. icterohaemorrhagiae, Lepto. grippotyphosa, Lepto. hyos and Lepto. pomona were used as antigens. All sera were stored at -0°C and tested at least three times by agglutination-lysis.

At the end of each of three experiments, when all the infected animals had been sacrificed, all the samples of serum collected during the investigation were subjected to a final test, in which the appropriate antigen was provided by a 5-8 days old culture of suitable visual density, with results that are later presented in the form of graphs.

Routine haematological examination comprised total red cell, total white cell, and differential leucocytic counts as well as an estimate of the concentration of haemoglobin. Thoma pipettes and a haemocytometer with Neubauer ruling were employed for the red and the white cell counts. The differential white cell count was made on films stained by the Leishman procedure and Sahli's method was used for the estimation of haemoglobin.

3. RESULTS.

The investigation was began by establishing that the serum of all the experimental animals was free from antibodies to the five leptospiral serotypes selected for this work.

work./

Compared with the figures given by Venn, quoted by Reddie (1946), in a few animals the red cell count was slightly higher than normal and exceeded 7 million cells per cubic millimetre. With the exception of piglets Nos. 20, 23, 24 and 26 which had about 10,000 cells per cubic millimetre, the total white cell count was rather on the low side and ranged from 5,000 (piglet No. 4) to 8,800 (piglet No. 21) leucocytes per cubic millimetre. Differential white cell counts did not reveal any abnormality but haemoglobin also was on the low side. Details of haematology together with the body temperatures prevailing at the start of the investigation are presented in Table 20.

TABLE 20.

THE BLOOD PICTURE AND BODY TEMPERATURE OF PIGLETS BEFORE THEY WERE USED FOR EXPERIMENT.

Piglet:	Haematological examination of blood before infection.								
	Total white cell count 10-3	%age of Neutrophils	%age of Lymphocytes	%age of Monocytes	%age of Eosinophils	%age of Basophils	Red cell count 10-6	Haemoglobin 9/100 ml.	
1	6.0	52.0	38	5	4	1.0	6.0	7.4	102.4
2	5.4	61.0	32	5	2	-	5.3	8.1	102.6
3	5.3	63.0	31	3	3	-	5.4	6.8	102.8
4	5.0	56.0	36	5	3	-	4.7	7.0	102.5
5	6.8	46.0	48	3	3	-	4.6	8.4	103.2
6	6.9	51.0	40	5	4	-	6.0	7.3	103.0
7	6.9	50.0	43	4	2	1	5.6	6.7	103.2
8	8.0	58.0	37	3	2	-	7.0	9.0	102.8
9	6.3	52.0	42.0	3	3	-	5.3	7.7	102.6
10	5.9	56	32.0	3	2	-	7.6	8.7	102.2
11	5.8	51.0	43.0	3	3	-	6.7	[illegible]	102.8
12	6.0	53.0	39.0	3	5	-	7.[illegible]	8.1	102.2
13	5.4	59.0	36.0	4	1	-	7.[illegible]	8.4	102.6
14	6.8	50.0	45.0	3	2	-	5.6	8.7	102.0
15	6.0	50.0	45.0	2	3	-	5.5	8.8	102.3
16	8.7	50.0	45.0	2	3	-	5.1	7.7	102.4
17	8.3	55.0	42.0	2	1	-	6.3	7.3	103.0
18	7.0	49.0	44.0	4	2	1	5.7	7.4	103.0
19	6.0	45.0	49.0	3	3	-	5.5	7.7	102.6
20	10.0	54.0	42.0	2	2	-	5.1	8.1	102.0
21	8.8	50.0	43.0	4	2	1	5.4	7.3	102.8
22	6.3	49.0	46.0	3	2	-	5.6	8.4	102.7
23	10.0	52.0	43.0	2	3	-	5.0	8.6	102.2
24	10.2	52.0	42.0	4	2	-	5.7	7.4	102.6
25	8.3	53.0	41.0	3	3	-	5.5	8.1	103.0
26	10.0	56.0	40.0	2	2	-	5.5	8.5	102.8

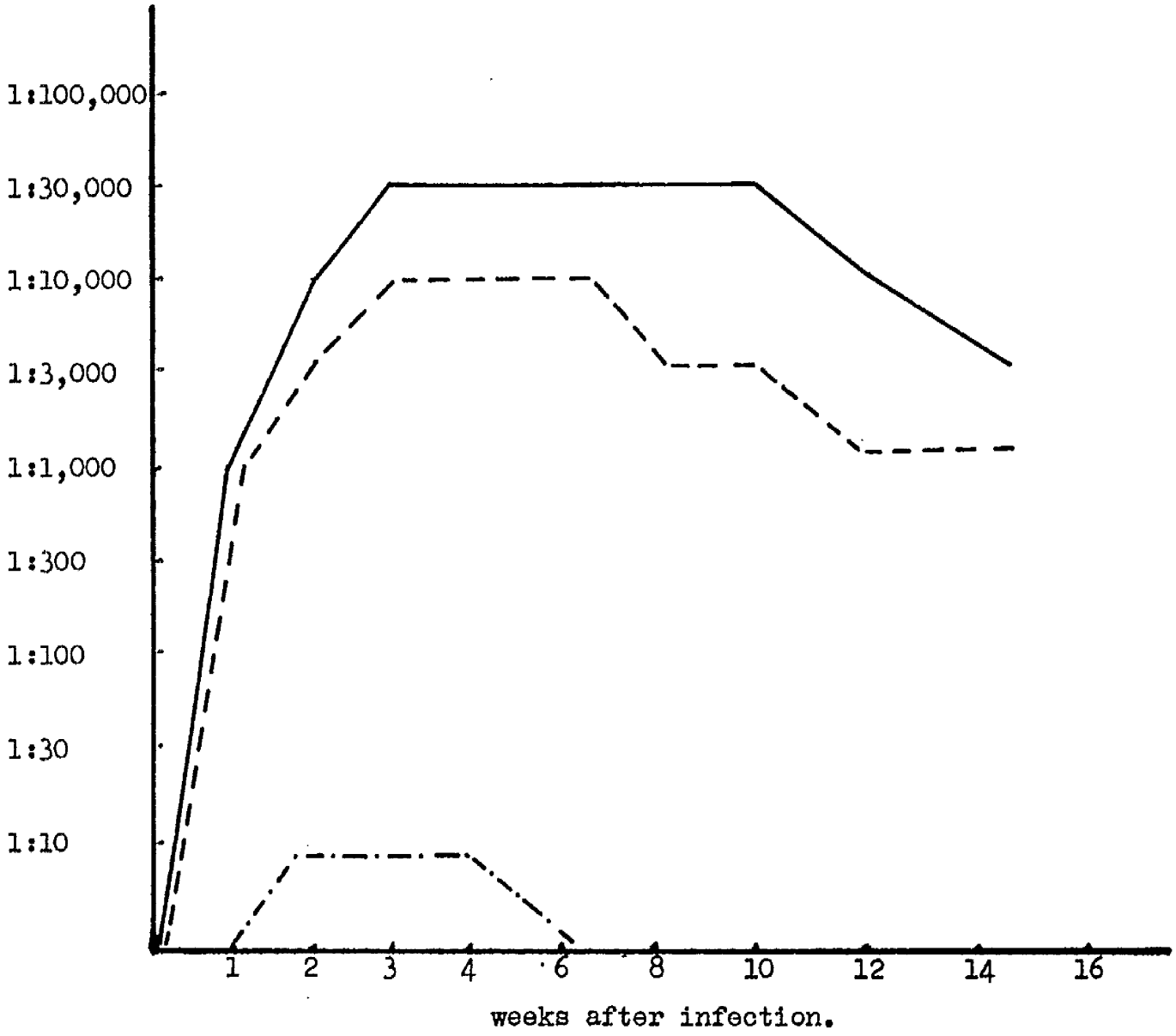


FIG. 6. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.1 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

The experimental findings in respect of each piglet were as follows:

FIRST EXPERIMENT.

Piglet No. 1.

The creature was infected on August 26, 1959 by means of subcutaneous inoculation of 1 ml. of a culture of Lepto. canicola, strain No. 35667. The first sample of serum, collected one week after infection, contained only a trace of antibody to Lepto. icterohaemorrhagiae but manifested a titre of 1:1000 to both the homologous and the Aldgate strains of Lepto. canicola. At the end of another week, the titre to the homologous organism rose to 1:10,000 but that to the Aldgate strain was only 1:3,000. Cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:10. A week later still, the highest level of titres was reached. With strain No. 35667 the titre was 1:30,000 and remained so throughout the next seven weeks, when it was found to have declined to 1:10,000 and finally to 1:3,000 in fifteen week's time from the day of infection. The Aldgate strain was agglutinated and lysed to a dilution of 1:10,000 by samples of serum obtained three, four and six weeks after infection. At the end of eight weeks the titre had dropped to 1:3,000, at which level it remained for two more weeks when it began to decline until at the end

end/

of twelve weeks it was 1:1000, which titre persisted until the end of experiment. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:10 occurred with sera procured at two, three and four weeks after infection. Tests with Lepto. pomona, Lepto. grippotyphosa and with Lepto. hyos proved negative. The serological response of this host is presented in Fig. 6 and the final titres are shown in Table 35.

Haematological examination revealed a very marked leucocytosis, inasmuch as the total white cell count rose from an original of 6,000 cells to 29,600 cells per cu. mm. at two weeks and 34,000 cells at ten weeks after infection. Thereafter, the number of white cells declined at 20,800 in twelve weeks and was 18,000 at the time of animal's death. That decrease coincided with a fall in the titre of leptospiral antibodies. During the second week of infection a slight increase of neutrophils was recorded and became more marked during the following week when it reached 56 per cent., at which level it remained for a further eight weeks before it gradually returned to the pre-infection level. There was neither a marked change in the number of lymphocytes nor any striking difference in the percentages of monocytes or of eosinophils. Prior to infection the basophil leucocytes were

TABLE 21.

EXAMINATION OF BLOOD.

PICLET No. 1. Infected on August 26, 1959.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
26.8.1959	6.0	52	38	5	4	1.0	6.0	7.4
2.9.1959	7.8	52	42	4	2	-	6.2	8.8
9.9.1959	29.6	53	42	3	2	-	6.9	8.5
16.9.1959	25.1	56	40	2	2	-	6.2	7.5
23.9.1959	27.5	55	42	2	1	-	6.7	8.4
7.10.1959	27.6	55	41	2	2	-	6.8	8.0
20.10.1959	33.0	56	40	2	2	-	6.5	8.4
3.11.1959	34.0	53	42	3	2	-	6.8	7.9
20.11.1959	20.8	52	42	4	2	-	6.8	8.4
7.12.1959	18.0	51	43	3	3	-	6.5	8.3

were/

present to the amount of 1 per cent. The red cell count ranged from 6-7 million erythrocytes per cu. mm., but haemoglobin estimations were rather on the low side and lay between 7.4-8.8 g. per 100 ml. Detailed haematological results are contained in Table 21.

At the time of infection the body temperature was 102.4°F. It rose slightly on the following day, reached 104°F. on the third day after infection and then dropped, to fluctuate between 102 and 103.2°F. and returned to normal. During the tenth week of the experiment a third rise was observed and attained 103.8°F., thereafter remaining at 102.2-103.2°F. until the animal died. The temperature chart is presented in Fig. 7. On the seventh day after infection, the abdominal skin and the sides of the thighs presented red, erythematous patches which were of different sizes and disappeared in response to pressure, only to return almost immediately after the finger was removed. Some were of pin-head size but others were up to 1 cm. in diameter and all were of irregular outline. The rash disappeared within a week. Otherwise the piglet was healthy. Transient leptospiruria was observed during the sixth week of infection.

Weekly blood-cultures into Schüffner's medium

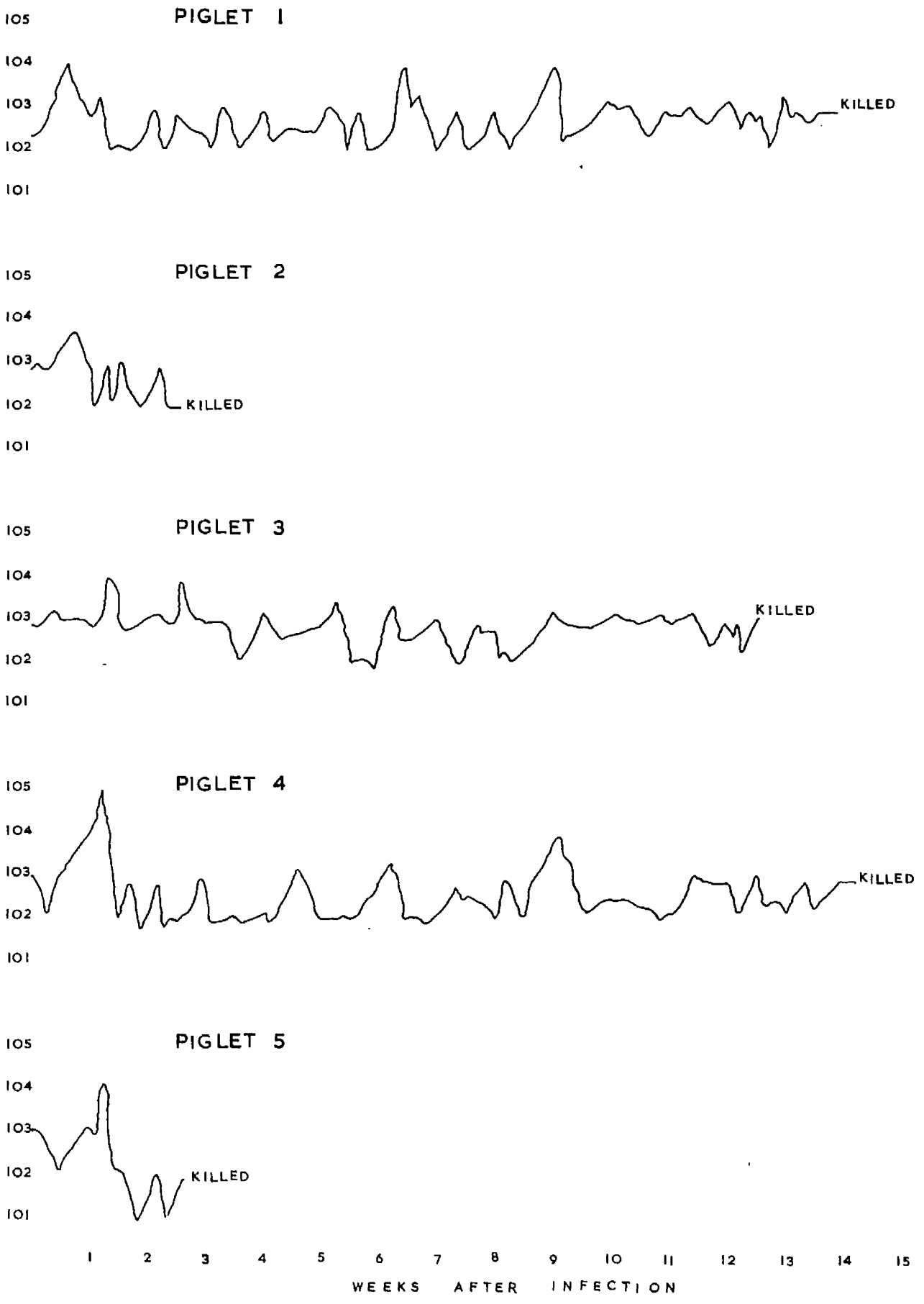


FIG. 7. TEMPERATURE CHARTS OF THE PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

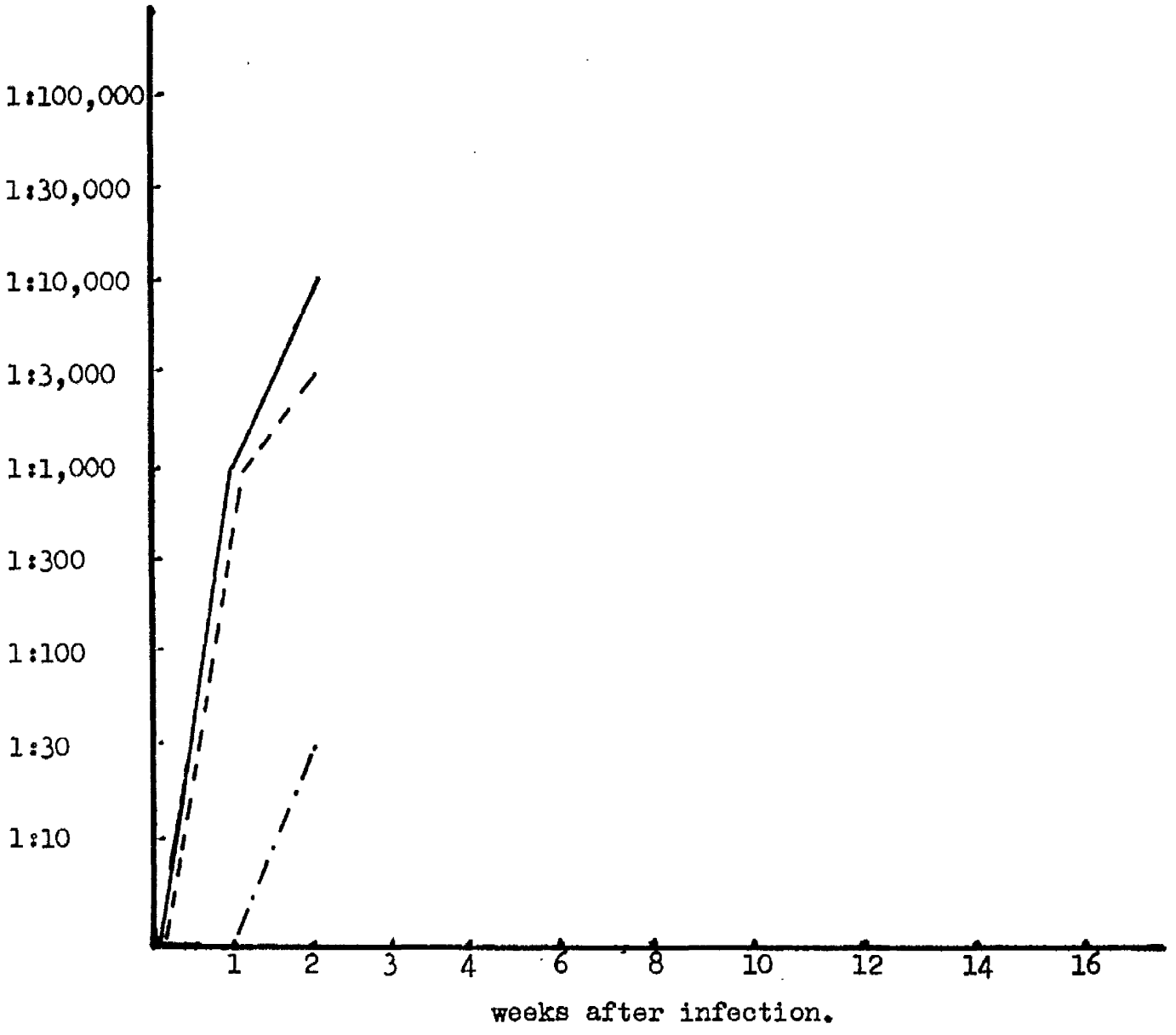


FIG. 8. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.2 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

medium/

remained sterile. The animal was sacrificed in 14 week's time and macroscopical lesions were not found at post-mortem examination.

Leptospiral growth occurred in half of the cultures made from kidney material and, on serological examination, the organism proved to be Lepto. canicola. Attempts to isolate the organism from the remaining viscera were unsuccessful.

Piglet No. 2.

An antibody response identical with that recorded for Lepto. canicola in piglet No. 1 was detected in the serum collected at one week and also at a fortnight after infection. Cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:30 but there was not any reaction with the other leptospiral antigens. Those findings are shown in Fig. 8 and in Table 35 as well. In the 14 days following infection, the total white cell count rose five-fold, namely, from 5,400 to 26,900 cells per cu. mm. There was an increase of neutrophils from 51 to 56 per cent., but monocytes dropped from 5 to 3 per cent. The percentage of eosinophils, the total red cell count and the amount of haemoglobin were within the normal range (Table 22).

The body temperature, which was 102°.6 F. at the time of the start of the experiment, rose to 103.6°F. by the

TABLE 22.

EXAMINATION OF BLOOD.

PICLET No. 2. August 26, 1959.
Infected on .....

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
26.8.1959	5.4	51	42	5	2	-	5.3	8.1
2.9.1959	5.8	53	41	3	3	-	5.9	7.7
9.9.1959	26.9	55	40	3	2	-	6.6	8.0

the/

fourth day, dropped to 102°F. two days later and persisted at 102-103°F. for the remainder of the creature's life (Fig. 7).

Aside from a rash, similar to that encountered in piglet No. 1, the animal was clinical normal. Microscopical examination by dark-ground illumination failed to reveal the presence of leptospira in a sample of urine collected two weeks after infection. At this stage of the experiment, the piglet was killed to allow of recovery, if possible, of leptospirae from internal organs other than the kidneys. Macroscopical lesions of the internal organs were not observed. Blood cultures made on the seventh and the 14th day after infection were negative but nine out of ten cultures inoculated with kidney tissue gave an abundant growth of organisms, which latter were subsequently proved to be Lepto. canicola. The media sown from the other internal organs remained sterile after 5 weeks of incubation, at which time they were discarded.

Dark-ground microscopical examination of films made from macerated kidneys revealed the presence of leptospirae.

Piglet No. 3.

A quite different antibody response occurred in

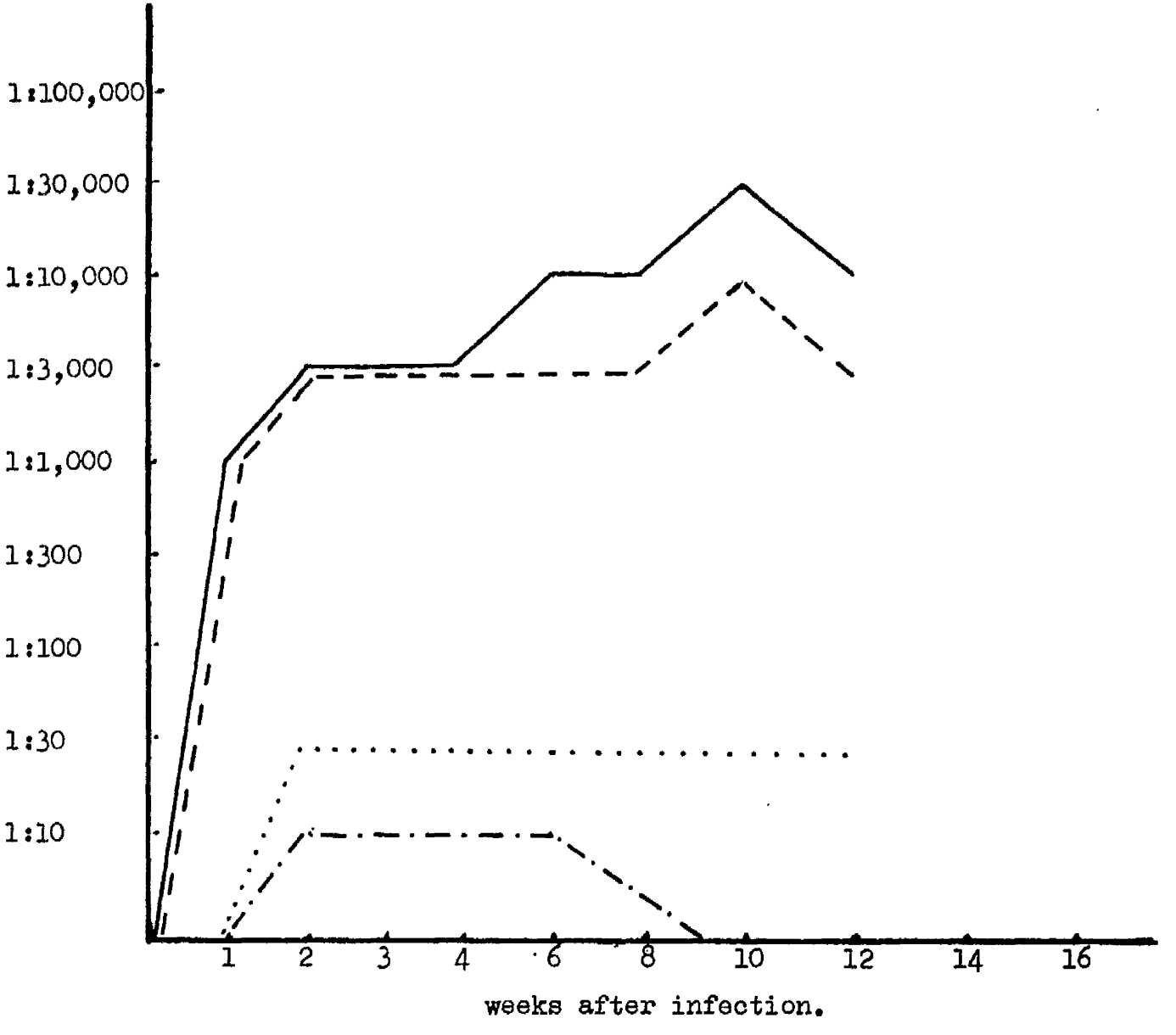


FIG. 9. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.3 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

in/

this host inasmuch as identical titres of antibody to both strains of Lepto. canicola were obtained during the first four weeks of experiment, at which time agglutination-lysis took place to a dilution of 1:3000. At the end of the sixth week the titre to strain No. 35667 had risen to 1:10,000, at which level it remained for a fortnight when it again increased to reach 1:30,000 two weeks later. Thereafter the titre declined to 1:10,000 at twelve weeks after infection. With the Aldgate strain the serum reacted to a dilution of 1:3,000 for two months after infection when the titre rose to 1:10,000 but had fallen to 1:3,000 when the animal was destroyed four weeks later. Cross-reaction with Lepto. icterohaemorrhagiae did not exceed 1:10 and was observable only in the samples of serum that were taken from the second to the sixth week of the experiment. Quite the most interesting finding was that the serum of this piglet gave a cross-reaction with Lepto. pomona to a dilution of 1:30. That reaction was observed with the serum obtained from the second bleeding and persisted to the end of the experiment. Reactions to Lepto. grippotyphosa and to Lepto. hyos were not recorded. The nature of the antigenic response in this case is illustrated in Fig. 9 and the final titres of antibody are included in Table 35. A gradual rise in the total white cell count, from 5,300 cells per cu. mm. before infection to 39,400

TABLE 23.

EXAMINATION OF BLOOD.

PIGLET No. 3. August 26, 1959.
Infected on .....

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
26.8.1959	5.3	51	43	3	3	-	5.4	6.8
2.9.1959	8.4	52	40	3	5	-	5.1	7.5
9.9.1959	24.5	53	40	3	4	-	6.8	7.4
16.9.1959	22.4	50	41	4	5	-	6.0	7.6
23.9.1959	25.3	56	40	2	2	-	5.6	7.3
7.10.1959	26.8	51	42	2	5	-	5.7	7.7
20.10.1959	39.4	52	42	3	3	-	6.8	8.8
3.11.1959	29.0	51	43	4	2	-	6.8	8.8
20.11.1959	25.2	52	42	4	2	-	6.6	8.0

39,400/

cells after eight weeks, is equivalent to an increase of nearly eight-fold. The differential white cell count revealed a marked neutrophilia together with an increased number of eosinophils but the total red cell count and the amount of haemoglobin remained normal. Haematological details are presented in Table 23. The thermal reaction was inconspicuous (Fig. 7). Apart from a slight rash, lasting for four days, the animal remained clinically sound.

Transient leptospiruria was detected during the seventh week of infection. When the animal was killed at three months after infection, lesions were not observable at post-mortem examination.

Out of ten cultures made from renal tissue Lepto. ~~canicola~~ developed in only one, the others becoming contaminated. All cultures from the other internal organs proved negative for leptospirae.

Piglet No. 4.

This animal was infected by scarification of the skin. The antibody response was feeble and at one week after infection the titre to the homologous strain was 1:100 while that to the Aldgate strain was 1:30. One week later, the

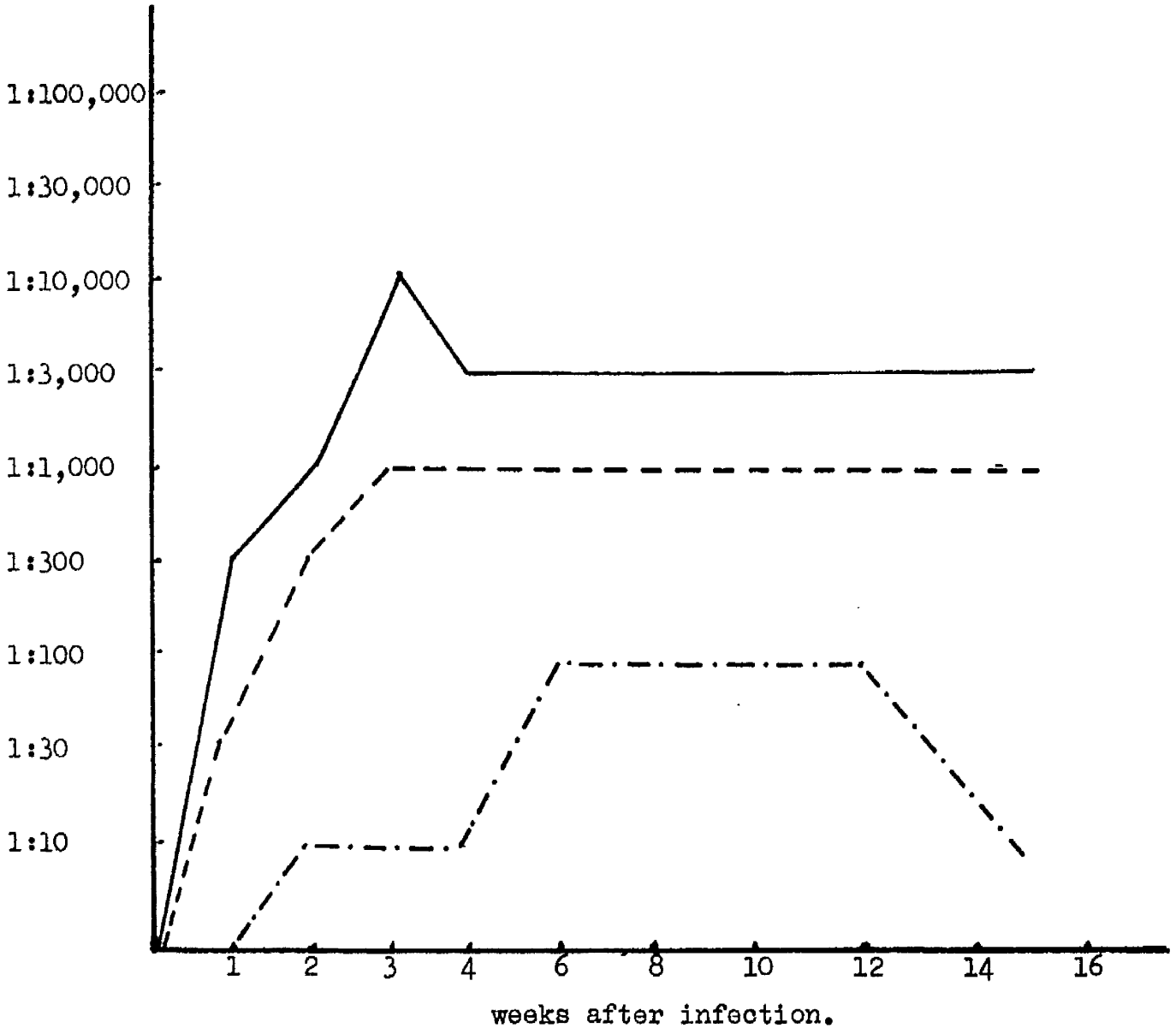


FIG. 10. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.4 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

the/

respective titres were 1:1000 and 1:300 and cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:10. The peak of leptospiral antibodies was reached three weeks after infection and was 1:10,000 to strain No. 35667 but only 1:1000 to the heterologous antigen. At the end of the fourth week of infection the titre of homologous antibodies had fallen to 1:3,000 but that to the Aldgate strain did not alter, which state of affairs remained until the animal's death. Cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:100 at six weeks after infection but by the end of the experiment that titre had fallen to 1:10. Reactions with Lepto. pomona, Lepto. aripoptyphosa and Lepto. hyos were not obtained (Fig.10 and Table 35).

The total white cell count rose from 5,000 cells per cu. mm. before infection to 28,700 at two weeks and to 32,000 at eight weeks after infection, when it gradually sank to 17,600 cells per cu. mm. on the last day of the experiment. The neutrophilic cells increased from 50 to 55 per cent. by the eighth week of infection but had fallen to 48 per cent. when the animal was sacrificed six weeks later. At the conclusion of the experiment, a monocytic rate of 2 per cent. was recorded and contrasted with a percentage of 5 so that was just obtained before infection.

TABLE 24.

EXAMINATION OF BLOOD.

PICLET No.4. Infected on August 26, 1959.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./100 ml.
26.8.1959	5.0	50	42	5	3	-	4.7	7.0
2.9.1959	8.8	52	41	4	3	-	5.0	8.1
9.9.1959	28.7	52	42	4	2	-	6.0	8.5
16.9.1959	26.9	53	40	4	3	-	6.4	7.8
23.9.1959	26.2	52	42	3	3	-	5.4	7.6
7.10.1959	27.0	52	44	2	2	-	6.5	7.5
20.10.1959	32.0	55	37	4	3	-	6.0	7.6
3.11.1959	20.0	54	41	2	3	-	6.0	8.4
20.11.1959	19.8	49	45	2	4	-	6.3	8.2
7.12.1959	17.6	48	47	2	3	-	6.2	8.5

infection./

The percentage of eosinophils was scarcely altered. The total red cell count varied from 4.7-6.5 millions per cu. mm. and the level of haemoglobin was low, being from 7-8.5 g. per 100 ml. Those haematological changes are summarized in Table 24.

There was a pronounced thermal reaction inasmuch as by the seventh day of infection the body temperature had risen to 105°F. The temperature dropped to 102°F. in two days and thereafter undulated between 101.5 and 103.4°F. for the ensuing eight weeks. At that time another rise to 103.8°F. occurred and was succeeded by a period of fluctuation between 102 and 103°F. that lasted for the rest of the animal's life (Fig. 7). A cutaneous rash was noted but otherwide the animal remained clinically healthy.

As in the previous case leptospiruria was detected during the seventh week of infection. When the piglet was killed at the end of 15 weeks gross lesions were not identifiable at post-mortem examination. All cultures became heavily contaminated by bacteria and proved negative for leptospirae.

Piglet No. 5.

Infection was again effected by scarification of

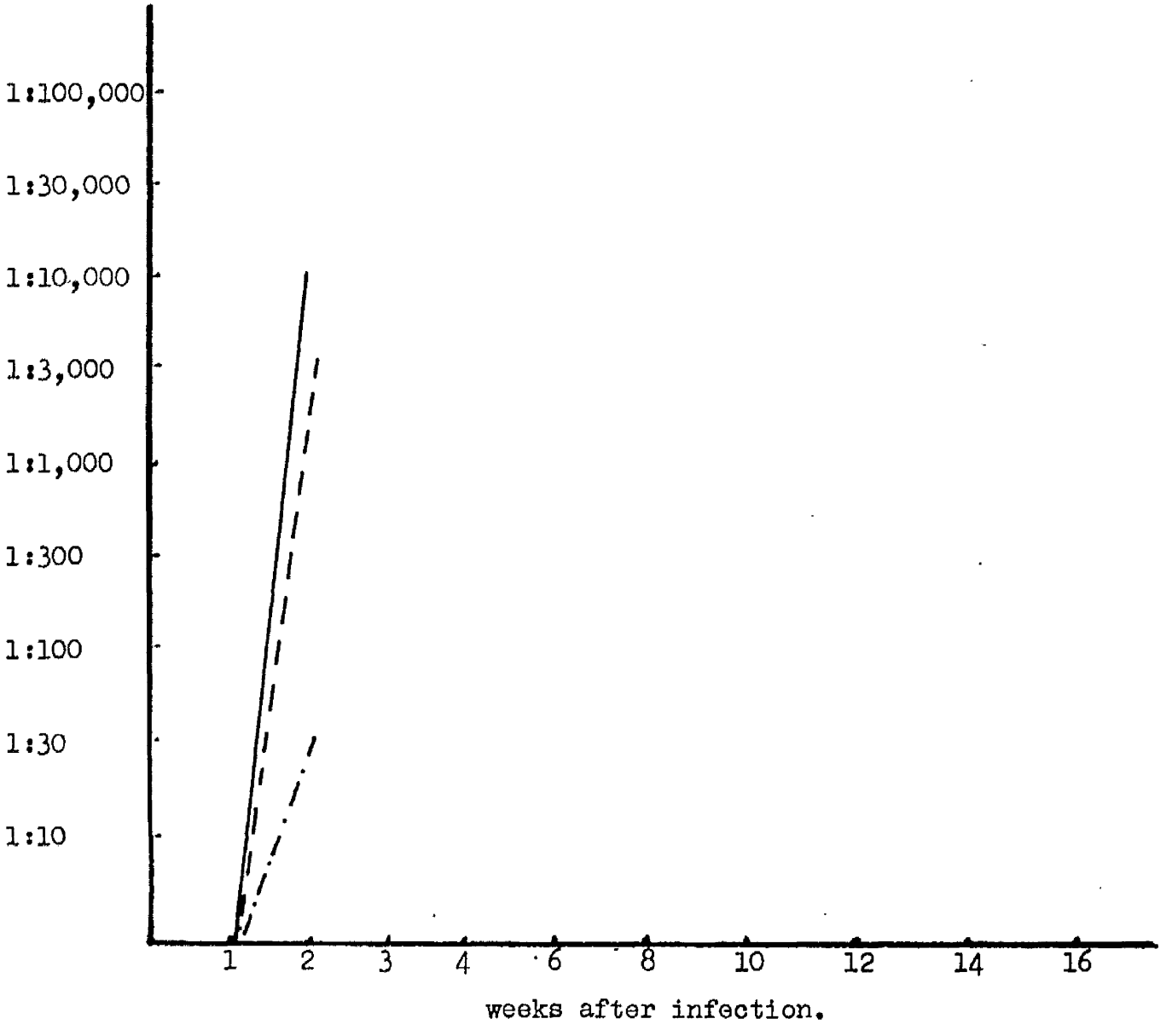


FIG. 11. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 5 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

of/

the skin. There was a delayed immune response and leptospiral antibodies were not detectable in the sample of serum that was taken at the seventh day of the experiment. This piglet was the only member of the group to yield leptospirae in culture made from the peripheral blood one week after infection and it may be that the absence of specific antibodies in the serum was responsible for the persistence of leptospiraemia for longer than usual. Thereafter there was a rapid rise of specific antibodies in the blood and by the end of the second week a titre of 1:10,000 to strain No. 35667 and of 1:3,000 to the Aldgate strain was forthcoming. Cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:30 (Fig. 11 and Table 35). Haematological examination revealed that the total white cell count rose from 6,800 prior to infection to 11,700 cells per cu. mm. at the end of the first, and to 23,000 cells at the end of the second, week of the experiment. The percentage of neutrophils also increased from 46 to 52 but the numbers of monocytes and of eosinophils were unaltered. As compared with the previous animals, there was little, if any, change in either the total red cell count or the amount of haemoglobin (Table 25).

As is shown by Fig. 7, the temperature of the body

TABLE 25.

EXAMINATION OF BLOOD.

PICLET No. 5. Infected on August 26, 1959.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./100 ml.
26.8.1959	6.8	46	48	3	3	-	4.6	8.4
2.9.1959	11.7	49	44	3	4	-	4.8	8.1
9.9.1959	23.0	52	42	3	3	-	6.3	7.4

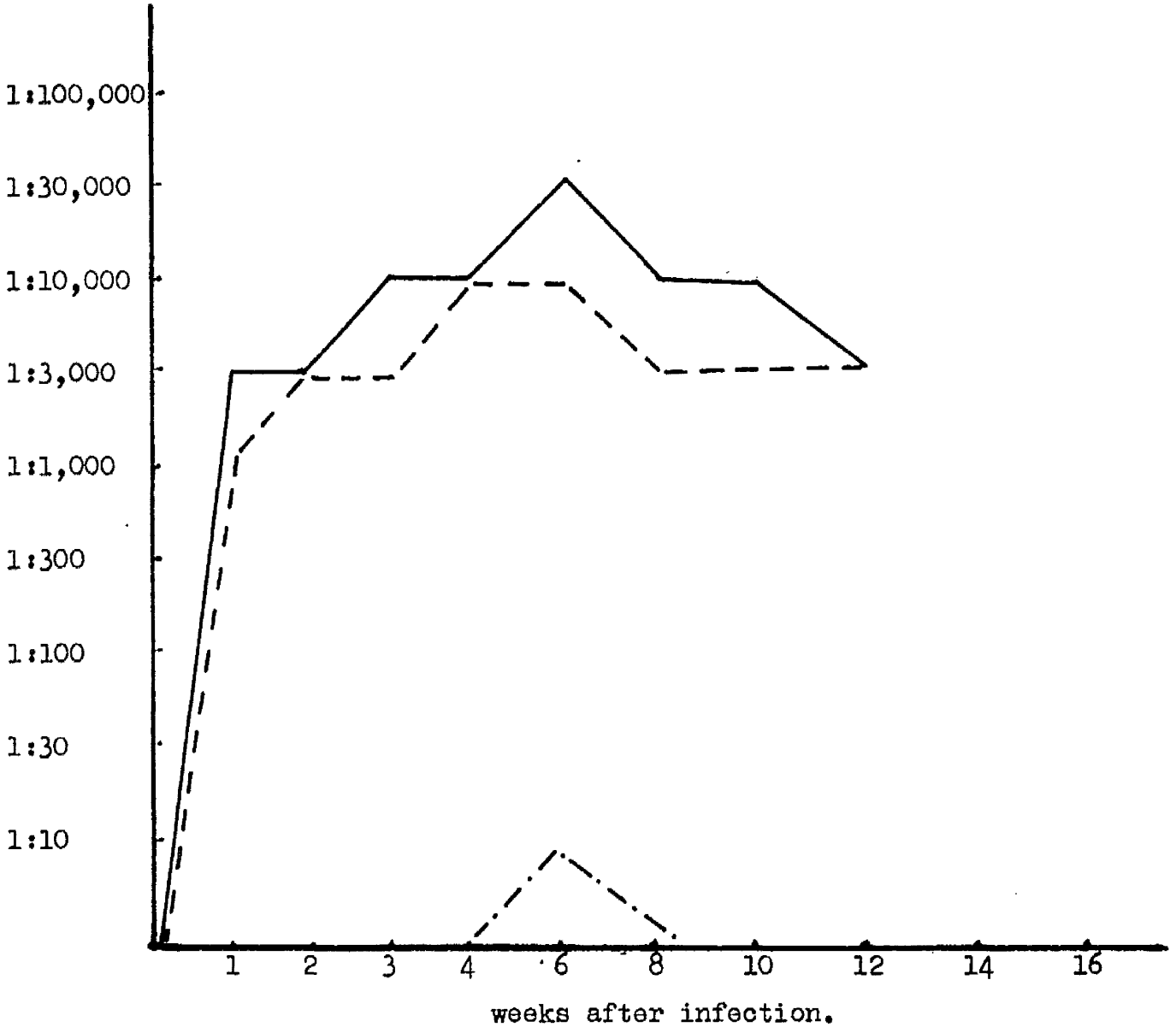


FIG.12. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 6 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

body/

rose to 104.2°F. by the seventh day after infection, dropped to 101°F. by the tenth day, and for the remainder of the experimental period, fluctuated between 101 and 102°F. Five days after infection diarrhoea was observable and lasted for four days. A cutaneous rash was scarcely conspicuous and leptospiruria was not detected. All blood cultures made seven days after infection yielded an abundant growth of leptospirae that was observable after one week of incubation. The piglet was killed two weeks after infection when post-mortem examination failed to reveal the presence of any gross lesions.

Lepto. canicola was recovered only from the renal tissue and all the cultures were characterized by heavy growth. Leptospirae were also demonstrable by microscopic means in films made from macerated renal tissue.

Piglet No. 6.

Scarification of the skin was the mode of infection. From Fig. 12 it will be observed that, during the first week of infection, the titre of antibodies to strain No. 35667 rose to 1:3,000 and that to the Aldgate strain of Lepto. canicola advanced to 1:1000. After another seven days, both titres were level at 1:3,000 but in the third week of

of/

infection the titre to strain No. 35667 increased to 1:10,000, ere it climbed further to 1:30,000 at six weeks. Two weeks later still the titre was down to 1:10,000 where it persisted for a fortnight when it fell to 1:3,000 at the time the creature was sacrificed, three months after infection. The titre to the Aldgate strain began to rise after the third week to reach a maximum of 1:10,000 which persisted for two more weeks before it returned to 1:3,000 at eight weeks after infection. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:10 was encountered only once, namely, in the case of the sample of serum collected six weeks of infection. As indicated in Table 35, reactions with Lepto. pomona, Lepto. gripotyphosa and Lepto. hyos did not occur.

Haematological examination revealed a leucocytosis which obtained throughout the whole experimental period but was maximal at six weeks after infection when the titre of antibodies was also at its highest. Thereafter, the number of white corpuscles slowly fell but was still 24,200 per cu. mm. when the animal was killed. There was little increase in the percentage of neutrophils and, as in most of the other experimental piglets of the group, the remaining haematological estimations approximated the normal (Table 26).

TABLE 26.

EXAMINATION OF BLOOD.

PICLET No.6. August 26, 1959.
Infected on .....

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
26.8.1959	6.9	50	41	5	4	-	6.0	7.3
2.9.1959	16.0	50	45	3	2	-	5.6	7.4
9.9.1959	25.2	53	43	2	2	-	5.4	8.4
16.9.1959	22.3	51	42	4	3	-	6.3	8.0
23.9.1959	26.6	51	45	2	2	-	6.8	7.4
7.10.1959	29.3	53	42	3	2	-	6.8	7.9
20.10.1959	26.6	52	45	2	1	-	6.5	7.7
3.10.1959	25.0	50	45	2	2	1	7.0	8.6
20.10.1959	24.2	51	43	3	3	-	6.9	7.3

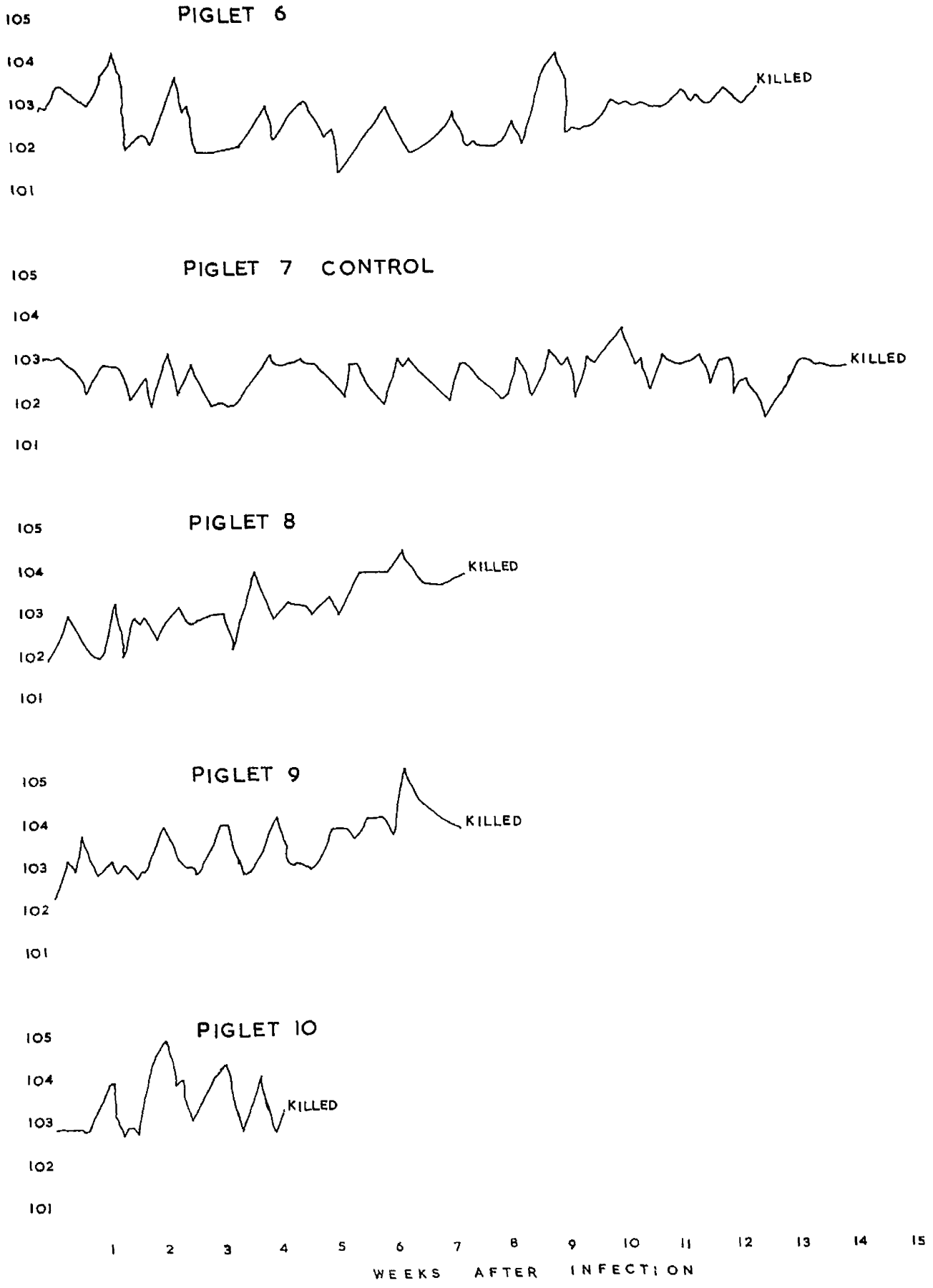


FIG. 13. TEMPERATURE CHARTS OF THE PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

(Table 26)./

The chart, given in Fig. 13, indicates a rise of body temperature to 104.2°F. on the seventh day of infection, followed by a sharp fall to 102°F. during the ensuing 24 hours. Throughout the next eight weeks the temperature fluctuated between 101 and 103.6°F. ere it rose again to 104°F. at the end of the ninth week. Thereafter the temperature remained between 102.2 and 103.3°F. Apart from a distinct rash, which appeared at the usual sites one week after infection to disappear within another week, the animal remained clinically healthy. Leptospirae were not demonstrated in the urine. The piglet was destroyed twelve weeks after infection and at post-mortem examination macroscopical lesions were not observable.

All cultures became contaminated by coliform organisms and Lepto. canicola was not recovered from the kidneys.

Piglet No. 7. Control.

This animal was kept for the purpose of control and, throughout the whole experiment, leptospiral antibodies were not demonstrable in its serum (Table 35). During the first fortnight of observation white cell counts of 6,900 cells per cu. mm. were recorded but during the following

TABLE 27.

EXAMINATION OF BLOOD.

PICLET No. 7. Control.
Infected on .....

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
26.8.1959	6.9	50	43	4	2	1	5.6	6.7
2.9.1959	6.9	50	45	2	2	1	6.0	7.2
9.9.1959	7.4	51	44	2	2	1	6.2	7.6
16.9.1959	8.2	50	44	3	3	-	6.4	7.5
23.9.1959	12.0	50	44	3	3	-	6.1	7.3
7.10.1959	14.0	50	43	4	3	-	6.6	7.8
20.10.1959	18.0	52	42	3	3	-	6.1	7.6
3.11.1959	18.0	50	45	3	2	-	6.0	7.5
20.11.1959	16.0	52	42	3	3	-	6.8	8.4
3.12.1959	15.0	50	44	3	3	-	6.8	8.2

following/

three weeks the count rose to 12,000. A further increase to 18,000 cells per cu. mm. occurred one month later and lasted for a fortnight before it declined gradually to a figure of 15,000 which obtained at the time of slaughter. That leucocytosis may have been caused by a non-suppurative inflammatory reaction that developed at the site of venipuncture after the sixth bleeding. The percentage of neutrophils and of lymphocytes remained within the normal range as did also the total red cell count and the amount of haemoglobin. Basophils were present to 1 per cent. but only during the first three weeks of the experimental period (Table 27).

Body temperature fluctuated between 102 and 103.2°F., but once fell to 101.4°F. and, on one occasion too, reached 103.6°F. (Fig. 13).

Leptospirae were not found in the urine. Post-mortem examination proved negative as did efforts to cultivate leptospires from the internal organs.

SECOND EXPERIMENT.

All the piglets of this group, excepting the control animal, piglet No. 14, were infected by scarification of the skin, in the manner that has been previously described.

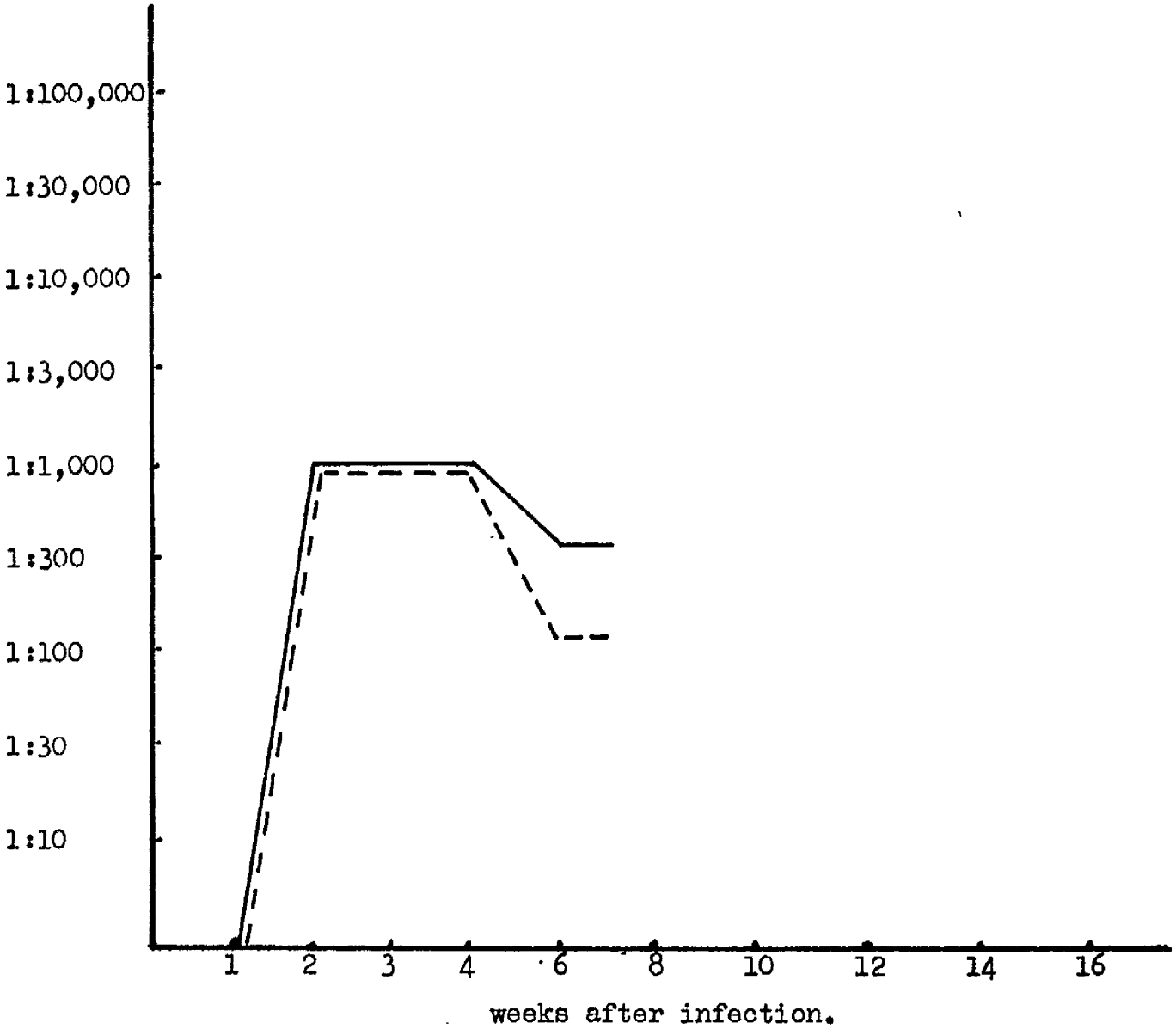


FIG.14. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.8 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

described./

Piglet No. 8.

Leptospiral antibodies were not demonstrable in the serum after one week of infection but the second sample of serum, secured seven days later, gave a positive reaction to both strains of Lepto. canicola to a dilution of 1:1000, which titre persisted for another two weeks. At six weeks the titre to strain No. 35667 had dropped to 1:300 whilst that to the Aldgate strain had declined to 1:100. Those levels were maintained until the animal was killed at seven weeks after infection. A reaction to Lepto. icterohaemorrhagiae, or to any of the other three leptospiral serotypes, was not recorded. The immunological response is presented in Fig. 14 and in Table 35.

A leucocytosis of 20,200 cells per cu. mm. was recorded four weeks after infection, following which the number of white cells had dropped to 18,200 at the time of killing. Only during the first week did the percentage of neutrophils increase while there was little change in the proportion of monocytes and of eosinophils. During the third to the fifth week of infection, the amount of haemoglobin fell to 7.5-7.9 g. per 100 ml. but recovered to 8.2 g. towards the end of experiment. The red cell count was unduly high, ranging from 6.0-7.6 millions

TABLE 28.

EXAMINATION OF BLOOD.

PICLET No. 8. Infected on February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	8.0	50	45	3	2	-	7.0	9.0
26.2.1960	10.4	53	41	3	3	-	6.3	8.1
4.3.1960	13.6	49	44	3	4	-	6.0	7.5
11.3.1960	13.1	49	45	4	2	-	7.2	7.7
18.3.1960	20.2	48	44	4	4	-	7.6	7.9
1.4.1960	18.2	50	44	3	3	-	7.2	8.2
7.4.1960	18.2	49	45	3	3	-	7.4	8.2

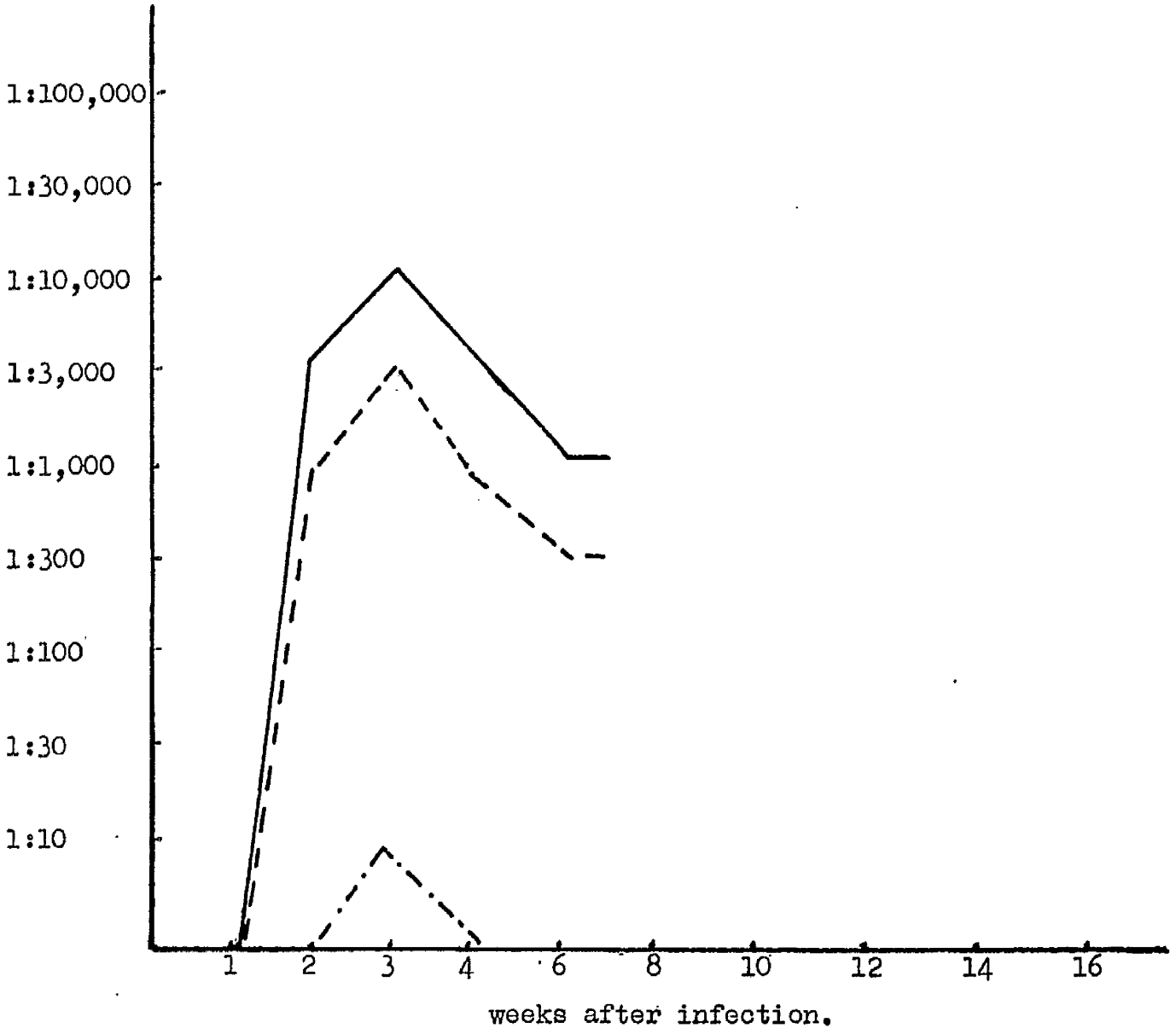


FIG.15. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 9 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

millions/

per cu. mm. (Table 28).

At the time of infection the temperature of the body was 101.4°F. and did not greatly exceed the normal limit throughout the experiment, save that a temperature of 104.4°F. was recorded during the sixth week (Fig. 13). A slight rash over the abdomen and on the inside of the thighs was noted on the third day after infection, but had disappeared five days later.

Leptospiruria was detected at four weeks after infection. Gross lesions were not exposed at post-mortem examination. All cultures became contaminated by other bacteria and Lepto. canicola was not recovered.

Piglet No. 9.

Leptospiral antibodies did not appear in the serum of this animal until the second week after infection when they reached a titre of 1:3,000 to the homologous organism and of 1:1000 to the Aldgate strain. By the end of three weeks, the respective titres were 1:10,000 and 1:3,000 and at six weeks they had fallen to 1:1000 and 1:300. The latter levels also obtained with the serum collected at slaughter. Cross-reaction with Lepto. icterohaemorrhagiae, to a dilution of 1:10, occurred only with the serum from the third bleeding (Fig. 15

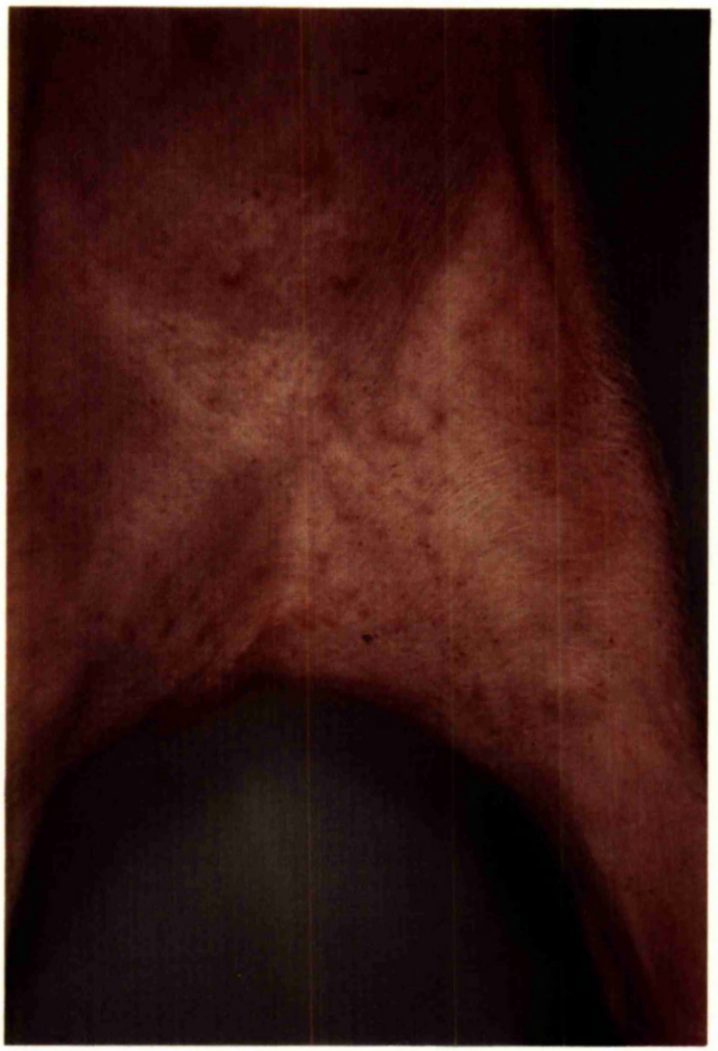


Fig. 16. Rash on the skin of the thigh and abdomen in piglet No. 9.

15/

and Table 35).

The total white cell count rose from 6,300 cells per cu. mm. before infection to a figure of 11,100 one week later. Thereafter, the count continued to increase to reach 18,000 cells at four weeks after infection but had dwindled to 14,000 when the experiment was ended. Little change was noted either in the percentages of neutrophils, lymphocytes, monocytes or eosinophils, or in the total red cell count or in the concentration of haemoglobin (Table 29). Throughout, the case was characterized by undulating fever with crests of 104°F. during the third and the fourth, and of 105.2°F. at the end of the sixth, weeks of infection. At the time of killing, however, the temperature of the body was down to 103.6°F. (Fig. 13).

Dermatitis together with conjunctivitis of a sero-mucoid type were noted seven days after infection. Next day a photograph of the cutaneous lesions was taken and appears in Fig. 16. Otherwise, the piglet remained healthy.

Leptospiruria was not encountered and gross lesions were not observed at post-mortem examination. Four days after infection, Lepto. canicola was recovered from the peripheral blood and, post-mortem, was obtained from renal tissue but not from any other part of the body.

TABLE 29.

EXAMINATION OF BLOOD.

PIGLET No.9.
Infected on February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./100 ml.
18.2.1960	6.3	52	42	3	3	-	5.3	7.7
26.2.1960	11.1	45	50	3	2	-	6.0	8.1
4.3.1960	14.0	46	48	4	2	-	6.0	8.4
11.3.1960	16.9	46	48	3	2	1	5.9	7.4
18.3.1960	18.0	49	45	3	3	-	5.6	7.9
1.4.1960	14.0	46	49	3	2	-	6.4	7.7
7.4.1960	14.0	48	46	3	3	-	6.4	7.7

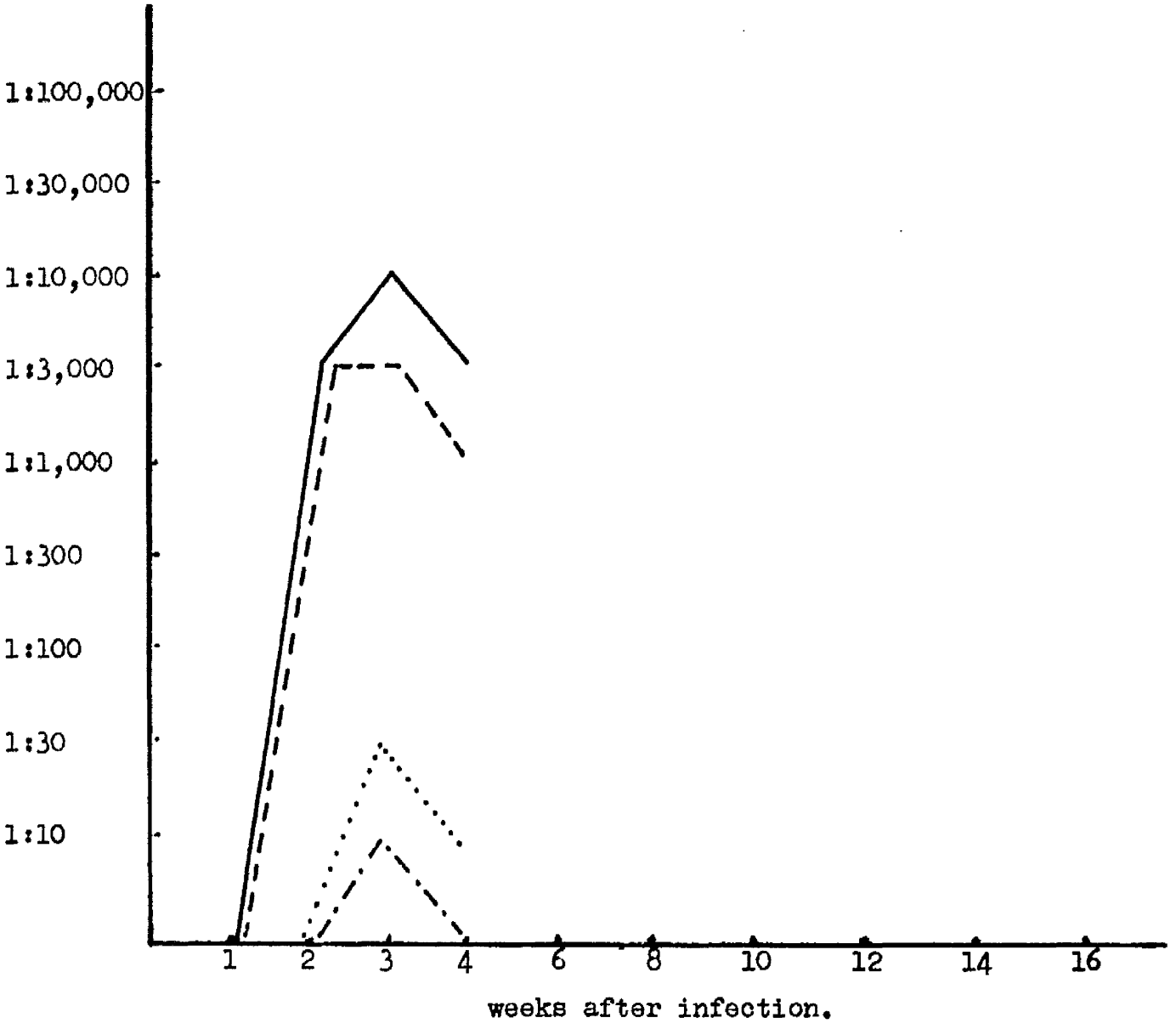


FIG.17. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.10. EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

body./

Piglet No. 10.

Fig. 17 shows that the titre of antibody rose rapidly during the second week of infection to a level of 1:3,000 in respect of both strains of Lepto. canicola. During the next seven days the titre to strain No. 35667 advanced to 1:10,000 but had fallen to the previously recorded level when the animal was killed at four weeks. On the other hand, the titre to the Aldgate strain remained at 1:3,000 until the end of the third week and had dropped to 1:1000 when the experiment ended. Cross-reactions with Lepto. icterohaemorrhagiae and with Lepto. pomona, to dilutions of 1:10 and 1:30, respectively, were observed in the case of the sample of serum collected three weeks after infection. At the time of killing, antibodies to Lepto. icterohaemorrhagiae were not detectable but Lepto. pomona was lysed at a dilution of 1:10 (Table 35). This is the second piglet to yield evidence of cross-reaction with Lepto. pomona.

A consistent increase of number of white cells was encountered throughout the experiment and varied from an initial 5,900 cells per cu. mm. to 20,000 at the time when the animal died. Apart from a slight rise at one week after infection, the proportion of neutrophils remained around 50 per

TABLE 30.

E X A M I N A T I O N O F B L O O D.

PICLET No. 10. Infected on February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	5.9	50	45	3	2	-	7.6	8.7
26.2.1960	11.9	57	39	3	1	-	6.4	8.2
4.3.1960	13.3	51	45	2	2	-	7.0	7.7
11.3.1960	14.6	49	45	3	3	-	6.9	8.4
18.3.1960	20.0	50	45	3	2	-	7.3	8.2

Fig. 18. Rash on the abdominal skin in piglet No. 10.

per/

cent. The percentage of lymphocytes varied between 39 and 45 and the proportion of eosinophils lay between 1 - 3 per cent., but the number of the monocytes was not altered. The red cell count was rather high and ranged from 6.4-7.6 millions per cu. mm. whilst the amount of haemoglobin was 7.7-8.7 g. per 100 ml. (Table 30).

At the time of infection the temperature of the body was 102.8°F. A maximum of 104.8°F. was recorded during the second week of infection, and thereafter the temperature varied between 103 and 104.4°F., ere it fell to 102.8°F. at the time of killing (Fig. 13). Fig. 18 is a photograph of the cutaneous rash that developed.

Leptospiruria was demonstrable at the end of the fourth week of the experiment.

At post-mortem examination macroscopic lesions of the internal organs were not encountered. Lepto. canicola was recovered from the blood on the fourth and the seventh days of infection and later from the renal tissue. All other cultures were negative.

Piglet No. 11.

Leptospiral antibodies did not appear in the serum

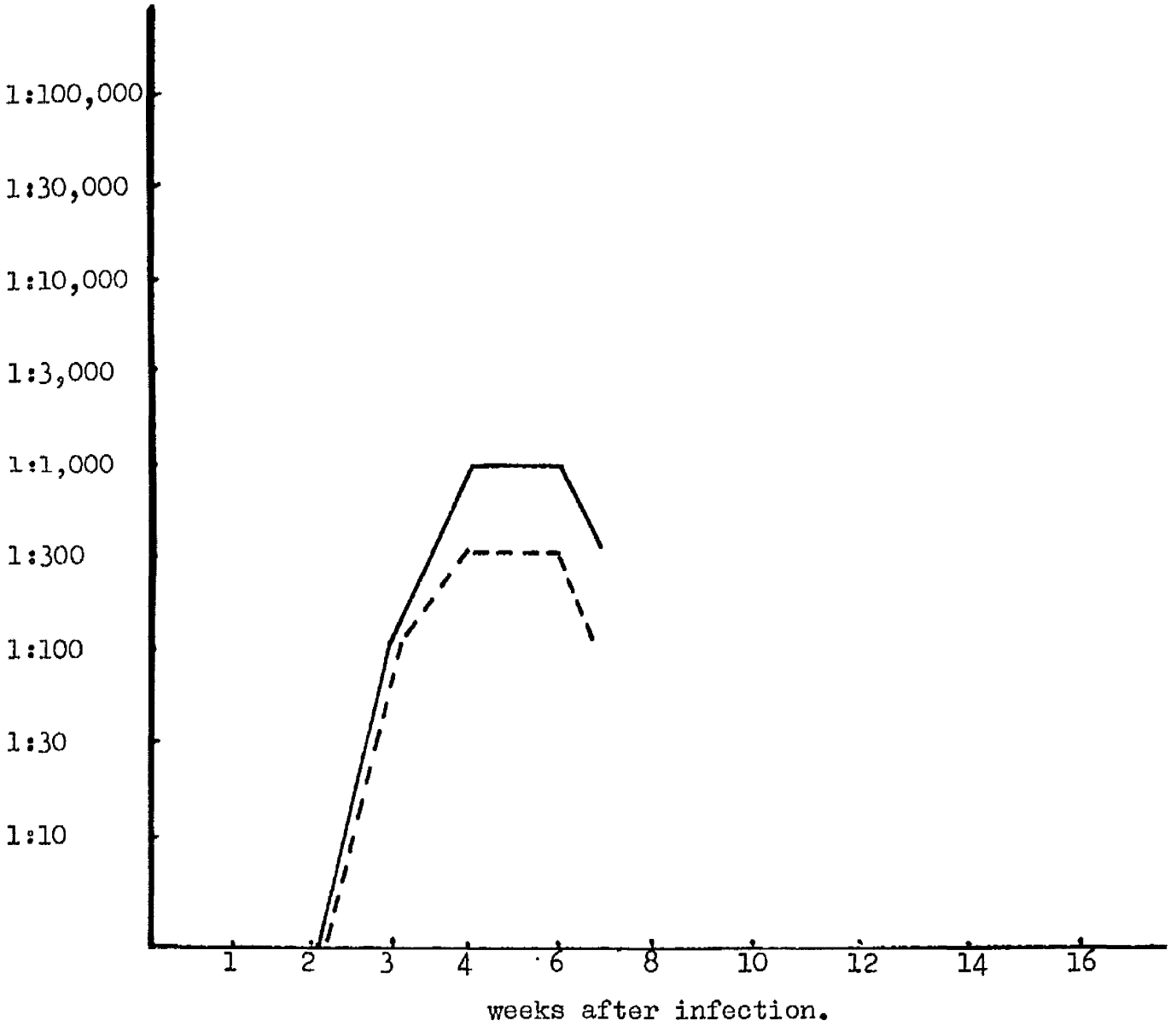


FIG. 19. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.11 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

serum/

until the third week after infection when the titre was 1:100 to both strains of Lepto. canicola. The titre to strain No. 35667 then rose to 1:1000 at the fourth week but had fallen to 1:300 a fortnight later. The highest titre to the Aldgate strain was 1:300 and was maintained from the fourth to the sixth weeks of infection, whereas at seven weeks it had declined to 1:100 (Fig. 19). Reactions with the other antigens were not encountered (Table 35). Haematological examination revealed a slow rise in the number of white cells to 22,000 cells per cu. mm. at the close of the sixth week, followed by a fall to 18,200 cells one week later. The neutrophils response was peculiar inasmuch as the percentage of those cells dropped from 51 at the start of the experiment to 43 at the end of the third week and thereafter returned to the normal level. The lymphocytes were increased to 50 per cent. at the end of the second week and remained in that proportion during the ensuing fortnight to fall to 43 per cent. after another week. The monocytic and the eosinophilic counts were but slightly changed and the red corpuscles amounted to 6.0-7.6 millions per cu. mm. The quantity of haemoglobin was low and ranged from 7.0-8.6 g. per 100 ml. (Table 31).

The thermal reaction presented in Fig. 20 indicates that a maximal temperature of 104.8°F. was recorded two weeks

TABLE 31.

EXAMINATION OF BLOOD.

PICLET No.11.
Infected on ..February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	5.8	51	43	3	3	-	6.7	8.4
26.2.1960	12.0	50	42	3	5	-	7.0	8.4
4.3.1960	13.5	45	50	2	3	-	6.3	8.6
11.3.1960	15.0	43	50	4	3	-	6.5	7.8
18.3.1960	17.2	45	50	2	3	-	6.0	8.6
1.4.1960	22.0	51	43	2	4	-	6.4	8.3
6.4.1960	18.2	50	45	2	3	-	6.6	8.5

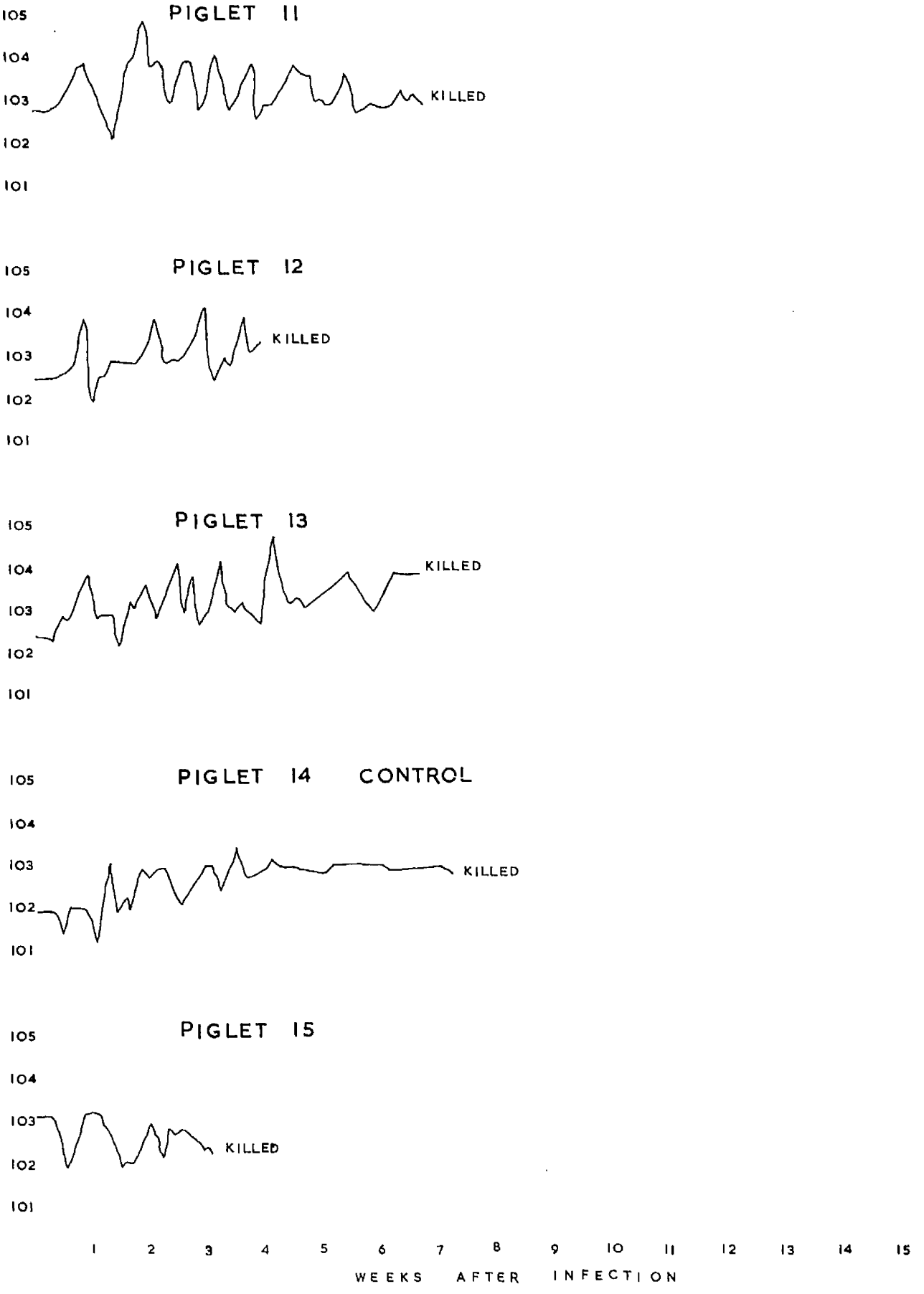


FIG. 20. TEMPERATURE CHARTS OF THE PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

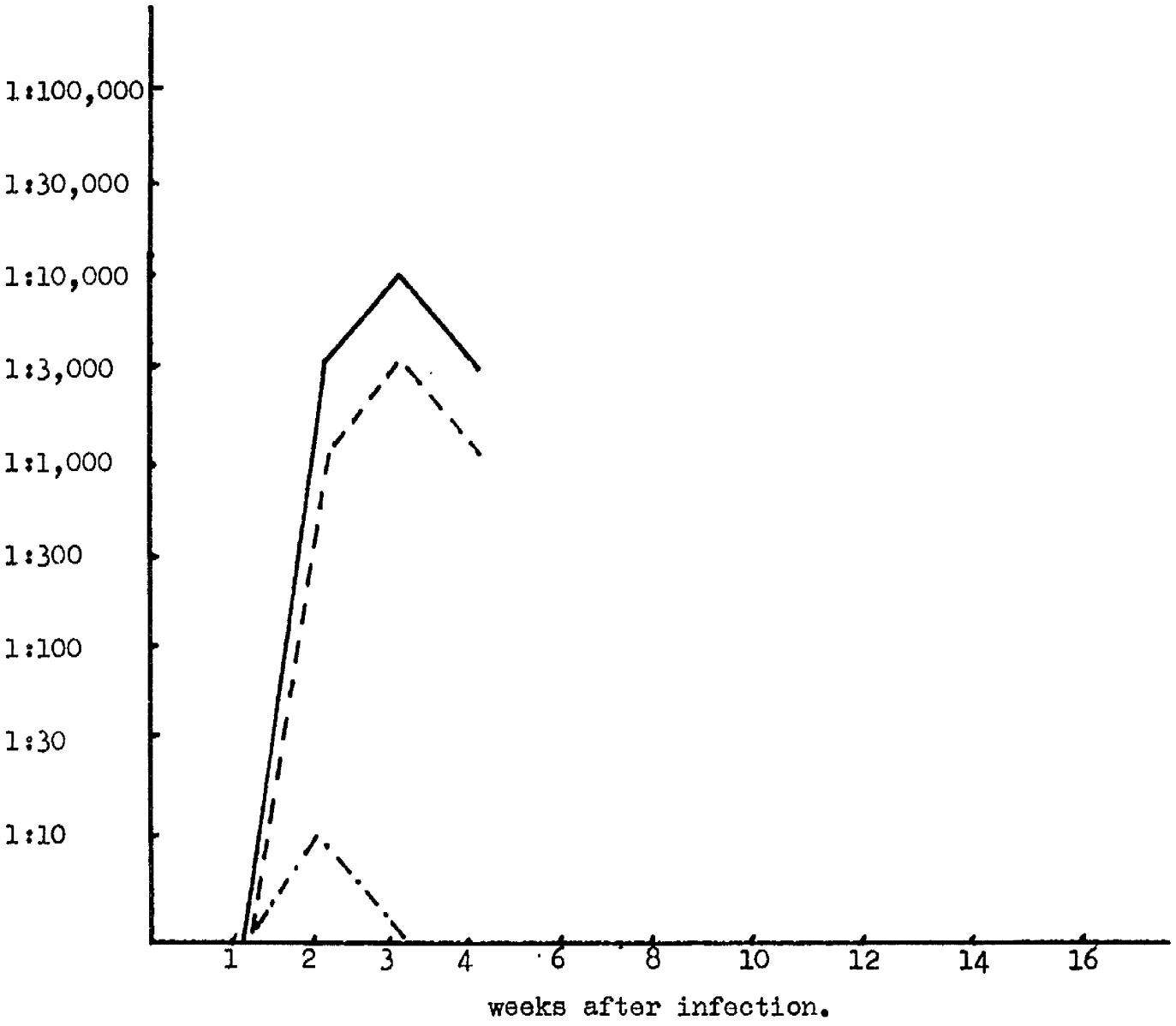


FIG.21. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 12 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

weeks/

after infection and that throughout the ensuing four weeks an appreciable degree of pyrexia was maintained.

An eruption of the skin, not unusual in distribution, appeared towards the end of the second week and lasted for four days.

Leptospiruria did not occur. Post-mortem examination failed to reveal the presence of any gross changes in the internal organs.

Lepto. canicola was recovered from the blood at four days after infection and subsequently from the renal tissue.

Piglet No. 12.

At the end of the second week the titre of leptospiral antibodies had risen to 1:3,000 with strain 35667 and to 1:1000 with the Aldgate antigen. One week later the respective titres had increased to 1:10,000 and 1:3,000 and, seven days later still, had fallen to the levels that obtained a fortnight previously. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:10 occured with the sample of serum taken at the end of second week. Fig. 21 illustrates the antigenic response of this host, while the final titres are given in Table 35.

TABLE 32.

EXAMINATION OF BLOOD.

PICLET No.12.
Infected on February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	6.0	50	42	3	5	-	6.5	8.2
26.2.1960	10.3	51	43	2	4	-	5.9	7.9
4.3.1960	15.0	54	39	3	4	-	6.0	7.7
11.3.1960	16.3	51	44	4	2	-	6.3	7.7
18.3.1960	18.2	47	47	4	2	-	5.6	7.9

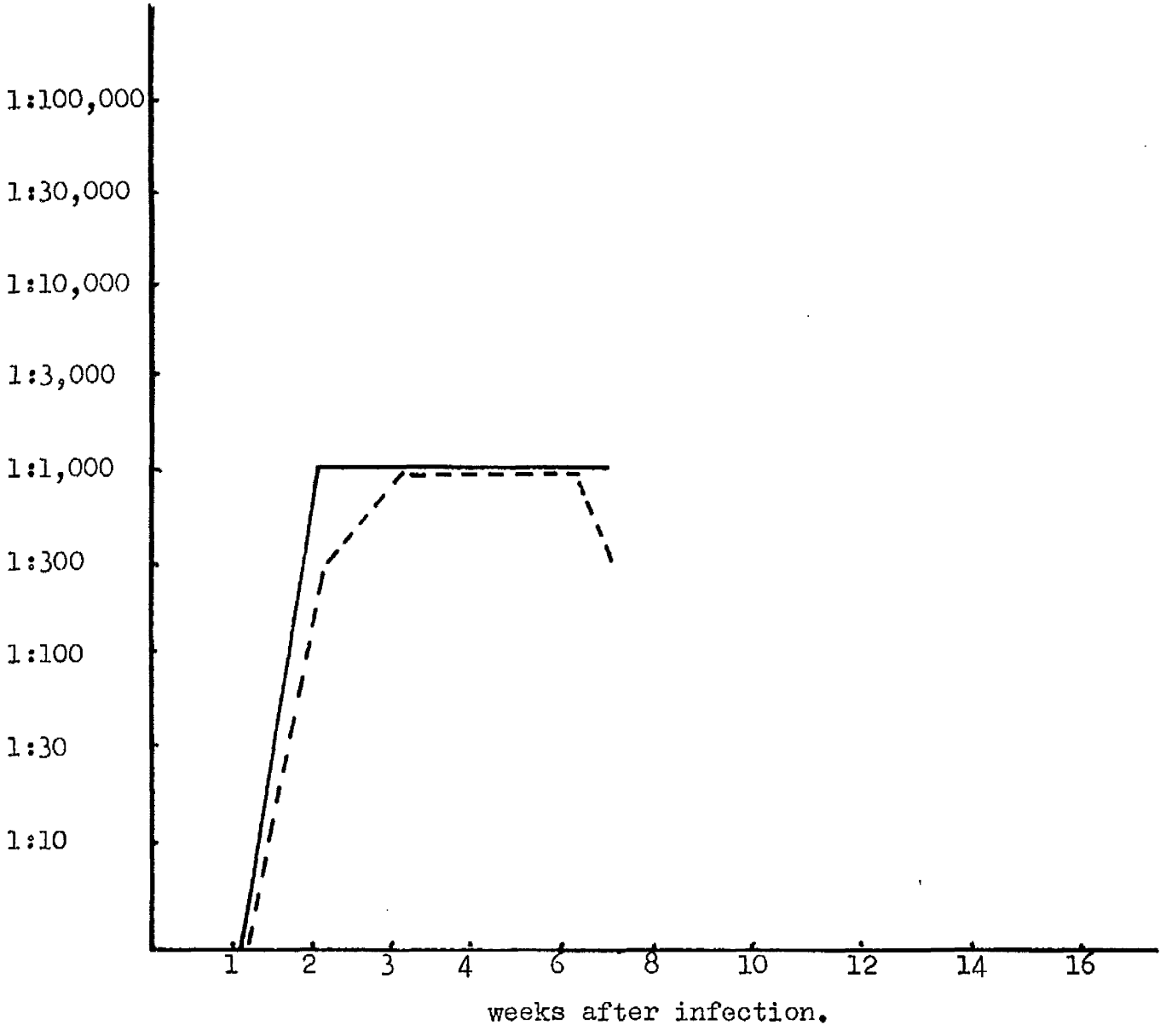


FIG. 22. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 13 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

Table 35./

During the four weeks following infection, the total white cell count was found to have risen from 6,000 to 18,200 cells per cu. mm. The percentage of neutrophils increased to 54 at the close of the second week but fell to 47 within the next 14 days. The percentage of monocytes was but little altered and the proportion of eosinophils varied from 1 - 5 per cent. The number of erythrocytes remained between 5.6 - 6.5 millions per cu. mm. and the amount of haemoglobin was 7.1 - 8.1 g. per 100 ml. (Table 32).

The first rise of body temperature took place five days after infection when it reached 103.8°F. and, after a sharp fall to 102°F., attained 104.2°F. at the end of the fourth week (Fig. 20).

Piglet No. 13.

Antibodies to the homologous strain of Lepto. canicola were detected in the serum from the second bleeding to a dilution of 1:1000 and remained at that level throughout the experiment. Antibodies to the Aldgate strain were demonstrable to a dilution of 1:300 at the end of the second week of infection, rose to 1:1000 a week later, to persist at that level for the ensuing three weeks, but had fallen to 1:300 at

at/

the time of animal's death. Reactions with the remaining antigens did not occur (Fig. 22 and Table 35). The total white cell count rose from an initial number of 5,400 cells per cu. mm. to 18,100 in the fourth week of the experiment, later to drop to 14,200. The percentage of neutrophils decreased from an original figure of 50 to 44 in the third week and thereafter lay between 45 and 47. At the time when neutrophils were at their lowest level, the proportion of lymphocytes had increased to 50 per cent. Monocytes fell from four to two per cent. but the eosinophils went up from one to five per cent. The red cell count was a little on the high side, namely, 6 - 7.4 millions per cu. mm. The quantity of haemoglobin varied from 8.2 - 8.9 g. per 100 ml. (Table 33).

The thermal reaction was slight but occurred at nearly weekly intervals, indicating an undulating type of fever. The first rise was to 103.8°F. and occurred on the fifth day after infection. Pyrexia of 103.6 - 104.2°F. was recorded 21 and 28 days respectively after infection and was maximal (104.6°F.) at the end of the fifth week. Thereafter the temperature fluctuated between 103.2 and 104.1°F. until the animal's death (Fig. 20).

Apart from a cutaneous rash the animal remained

TABLE 33.

EXAMINATION OF BLOOD.

PICLET No.13. Infected on February 18, 1960.

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	5.4	50	45	4	1	-	7.4	8.4
26.2.1960	14.9	48	47	3	2	-	7.3	8.7
4.3.1960	13.8	48	44	4	4	-	6.0	8.7
11.3.1960	16.0	44	50	3	3	-	6.0	8.8
18.3.1960	18.1	47	47	3	3	-	6.6	8.9
1.4.1960	14.2	45	50	2	3	-	6.8	8.2
7.4.1960	14.2	47	46	2	5	-	7.2	8.2

remained/

clinically normal.

Leptospiruria was demonstrated at the close of the fourth week. At post-mortem examination the internal organs appeared normal.

At four days after infection blood culture was negative but three days later all cultures were characterized by abundant growth of Lepto. canicola. Post-mortem, the organism was recovered only from the kidneys.

Piglet No. 14.

All serological tests of this control animal failed to reveal the presence of leptospiral antibodies. (Table 35).

White cell count remained within the normal range and there was little, if any, change in the percentages of neutrophils, lymphocytes or monocytes. The proportion of eosinophils, however, decreased from four to one per cent. The red cell count and the amount of haemoglobin also were normal (Table 34).

There was little alteration of the body temperature (Fig. 20); the skin was not affected and leptospirae were not found in the urine, whilst anatomical changes were not evident

TABLE 34.

EXAMINATION OF BLOOD.

PICLET No.14. Control.
Infected on .....

DATE OF BLEEDING	White count 10^3 per cubic mm.	Percentage Neutrophils	Percentage Lymphocytes	Percentage Monocytes	Percentage Eosinophils	Percentage Basophils	Red count 10^6 per cubic mm.	Haemoglobin gm./ 100 ml.
18.2.1960	6.8	50	45	3	2	-	5.6	8.7
26.2.1960	8.6	47	48	2	3	-	6.5	8.8
4.3.1960	7.8	48	47	2	3	-	6.2	8.4
11.3.1960	7.6	44	49	3	4	-	6.4	7.6
18.3.1960	10.0	50	46	3	1	-	6.0	8.4
7.3.1960	12.0	50	47	2	1	-	6.1	8.2

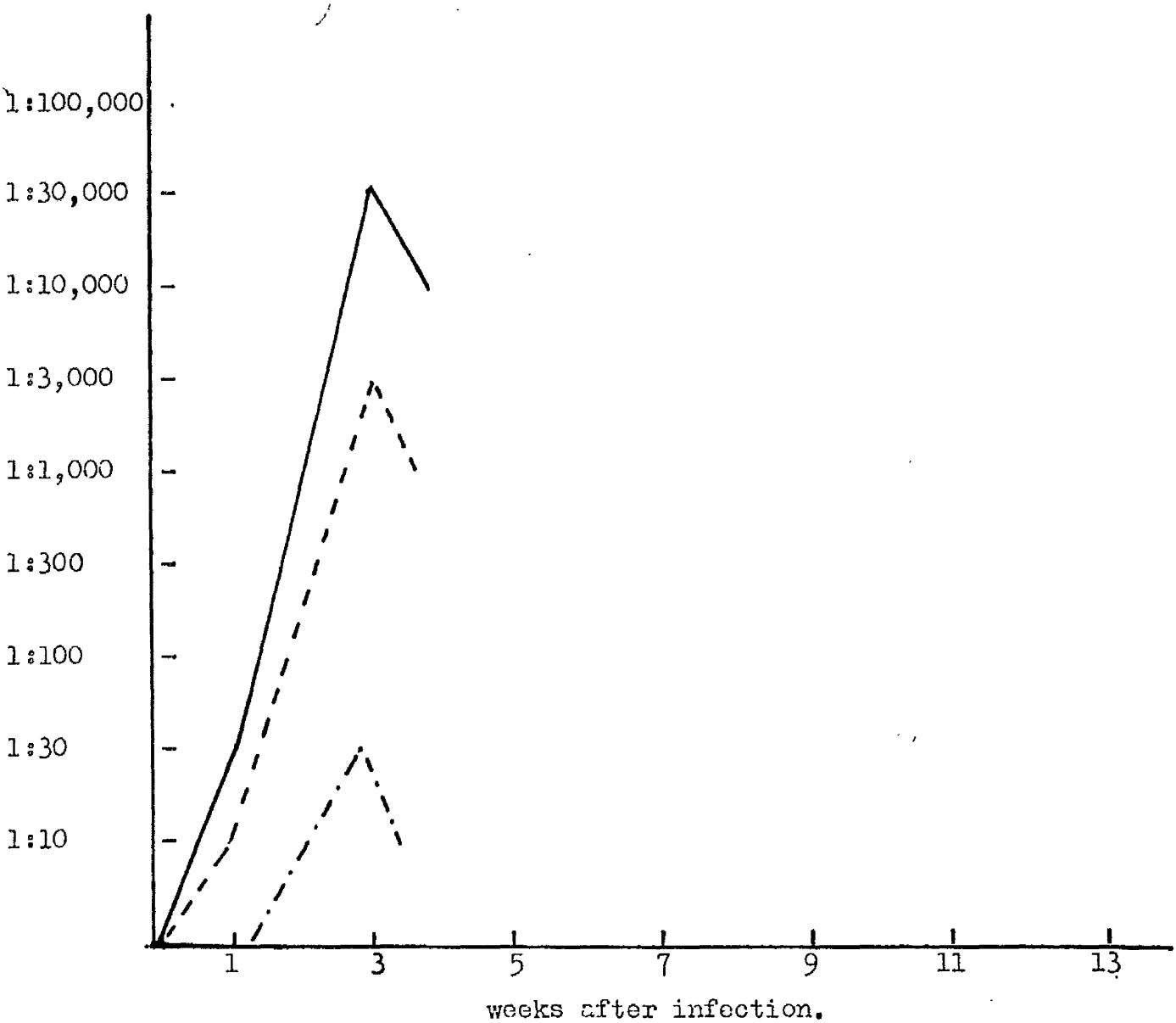


FIG. 23. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.15
EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —.—.—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

evident/

at post-mortem examination.

All cultures proved negative.

THIRD EXPERIMENT.

Because the results of the individual haematological examinations did not differ basically from those already recorded, detailed descriptions of each case and the relative Tables have been omitted.

Piglet No. 15.

One week after infection, a titre of 1:30 to the homologous strain and of 1:10 to the Aldgate strain of Lepto. canicola was established. Two weeks later the respective titres had increased to 1:30,000 and 1:3,000, and cross-reaction with Lepto. icterohaemorrhagiae also occurred to a dilution of 1:30. At the next bleeding, when the animal was killed, the corresponding titres were 1:10,000, 1:1000 and 1:10, as indicated in Fig. 23 and in Table 35.

The animal did not manifest fever at any stage (Fig. 20). An erythema, that appeared ten days after infection, was the only clinical abnormality.

Leptospiruria did not occur, but organisms were

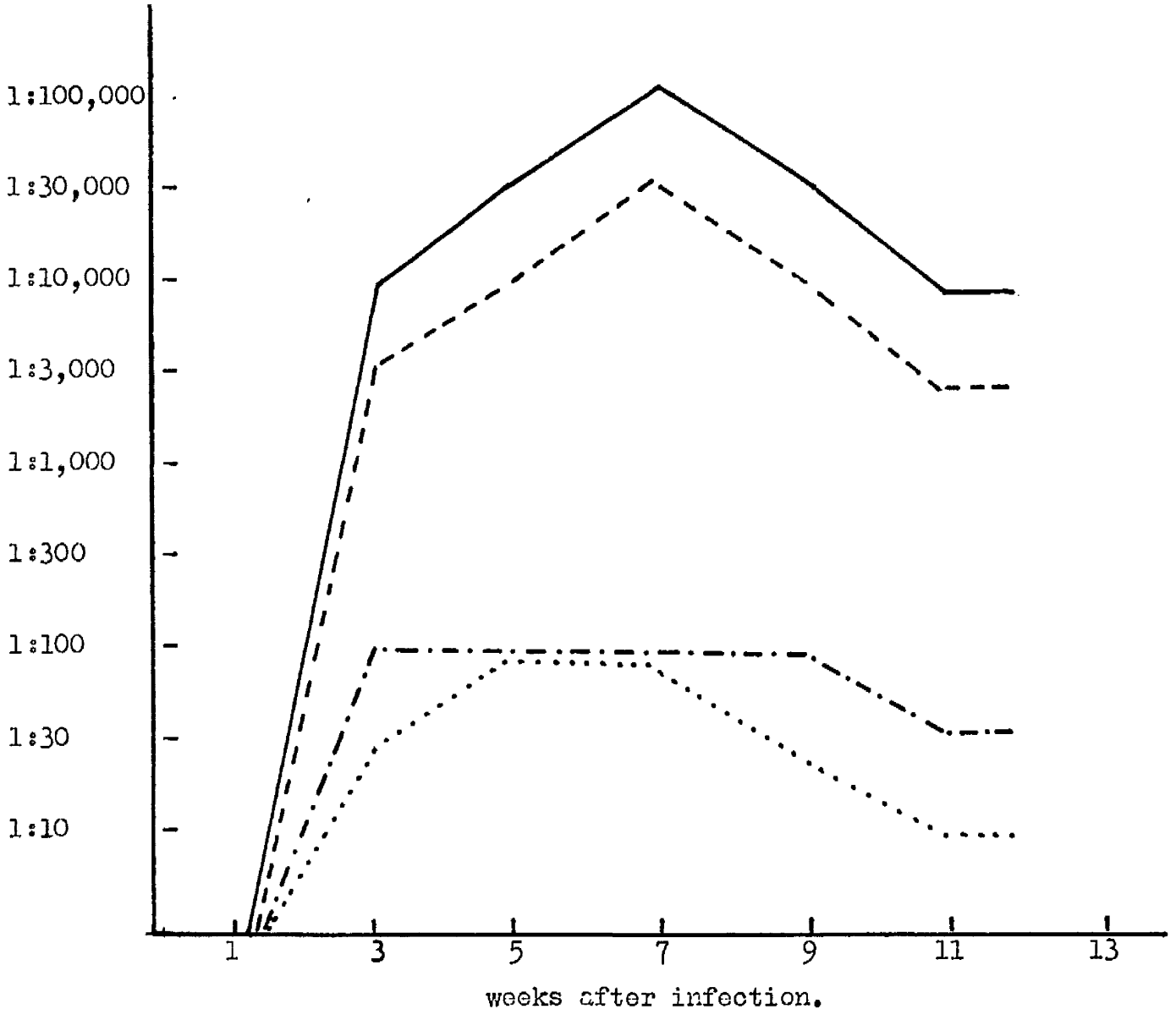


FIG. 24. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 16 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

were/

demonstrated microscopically in films made from macerated kidney tissue. Macroscopical lesions were not found at post-mortem examination. Blood culture was positive on the fourth, but not on the seventh, day. All cultures made from renal tissue yielded a heavy growth of Lepto. canicola but those from other organs of the body proved negative.

Piglet No. 16.

The agglutination-lysis test was negative at one week after infection but, by the end of the third week, antibodies were demonstrable to a titre of 1:10,000 with strain 35667 and to a dilution of 1:3,000 with the heterologous strain of Lepto. canicola. At the same time cross-reactions were observed to a dilution of 1:100 with Lepto. icterohaemorrhagiae and to 1:30 with Lepto. pomona. Two weeks later the titre to the homologous strain had risen to 1:30,000 and that to the Aldgate strain had increased to 1:10,000. At seven weeks, the titres were maximal at 1:100,000 and 1:30,000, respectively. Thereafter, the amount of antibody declined to levels of 1:10,000 and 1:3,000 which were recorded at the close of the eleventh week and persisted until the end of the experiment. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution

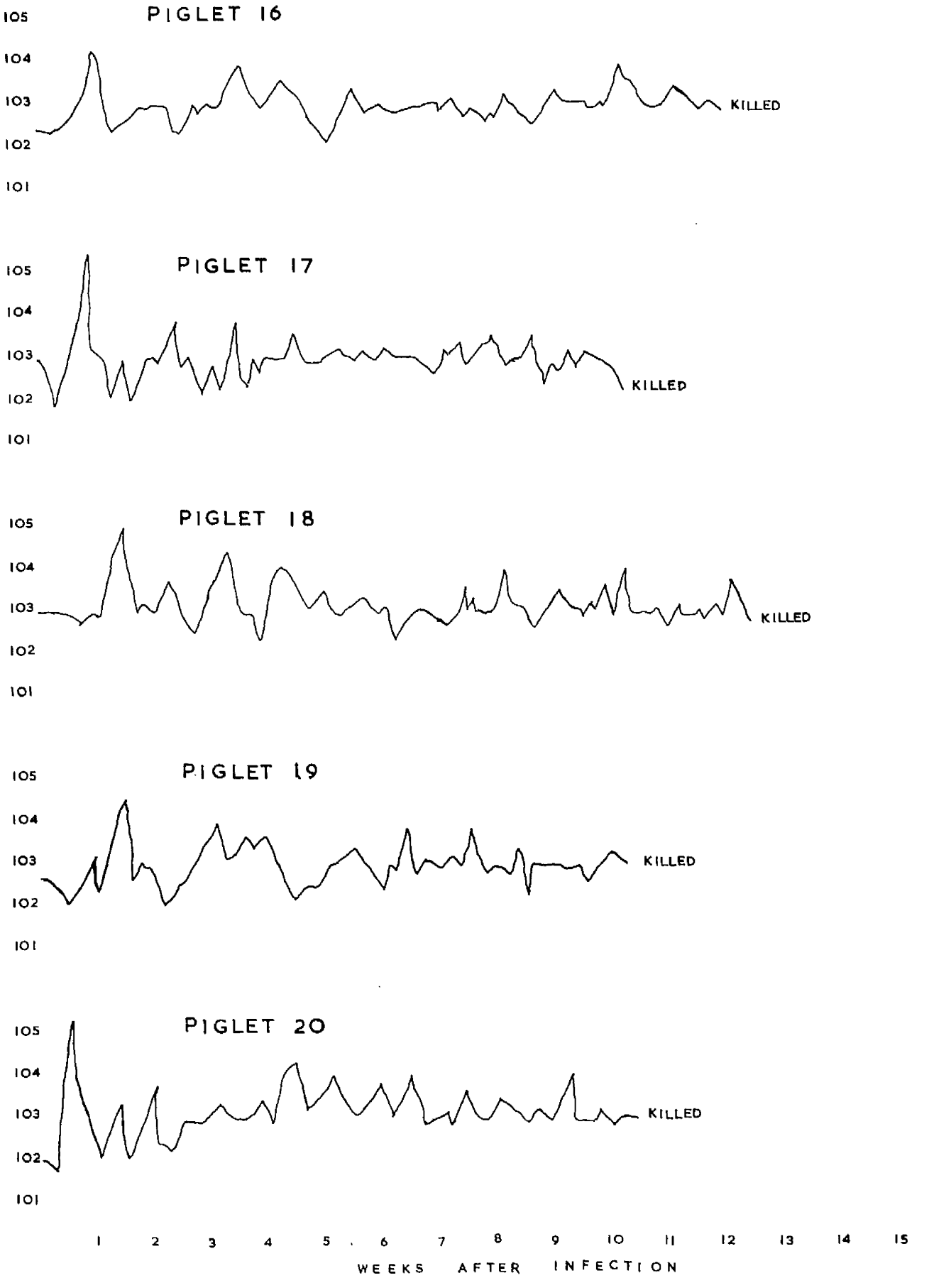


FIG.25. TEMPERATURE CHARTS OF THE PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

dilution/

of 1:100 continued to be demonstrable for two months, whilst the reaction with Lepto. pomona to a dilution of 1:100 obtained from the fifth to the seventh week ere it gradually fell to 1:10 at the end of the eleventh week. A reaction to Lepto. grippotyphosa or to Lepto. hyos did not occur (Fig. 24 and Table 35).

As shown in Fig. 25, the body temperature rose from 102.8°F. at the time of inoculation to 104.2°F. on the fifth day after infection. For the rest of the animal's life the temperature remained normal save for a slight rise to 103.8°F. during the third week of observation. One week after infection, a slight dermatitis developed but lasted for only four days.

Leptospiruria was not detectable until the twelveth week of experiment. Gross lesions were not found at post-mortem examination. Lepto. canicola was recovered from the blood four days after infection and later from kidney material but not from any of the other organs of the body.

Piglet No. 17.

One week after infection, antibodies were demonstrable in the serum but only in low titre, namely, 1:30 with

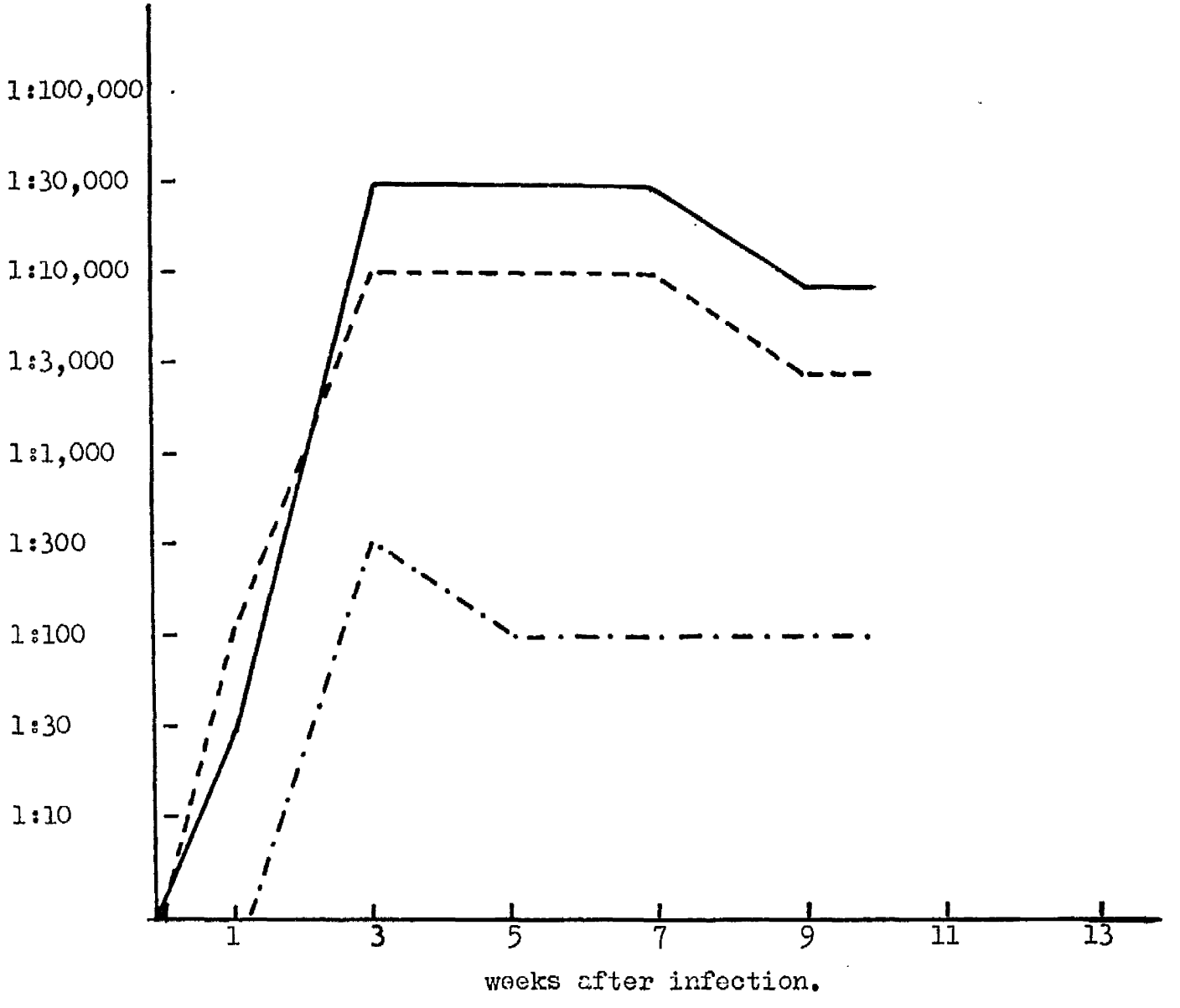


FIG. 26. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 17 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

with/

strain No. 35667 and 1:100 with the Aldgate strain of Lepto. canicola. During the next two weeks the titres rose to 1:30,000 and 1:10,000, respectively, and cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:300 also occurred. The amount of antibody to both strains of Lepto. canicola remained steady for four weeks ere it fell to 1:10,000 and 1:3,000, respectively, which levels were maintained until the animal was sacrificed. From the fifth week onwards, cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:100 continued to be manifest but there was not any reaction with Lepto. grippotyphosa or Lepto. hyos or Lepto. pomona (Fig. 26 and Table 35).

The thermal reaction is depicted in Fig. 25 and is characterized by a fall of body temperature from 103°F. to 101.6°F. on the first day after infection, followed by a rapid rise to a peak of 105.4°F. on the fifth day. During the remainder of the experiment the temperature of the body was normal except for moderate rises to 103.8°F. which occurred during the third and the fourth weeks of infection.

One week after infection the piglet lacked vigour and was not inclined to eat for about three days when slight but transient erythema appeared.

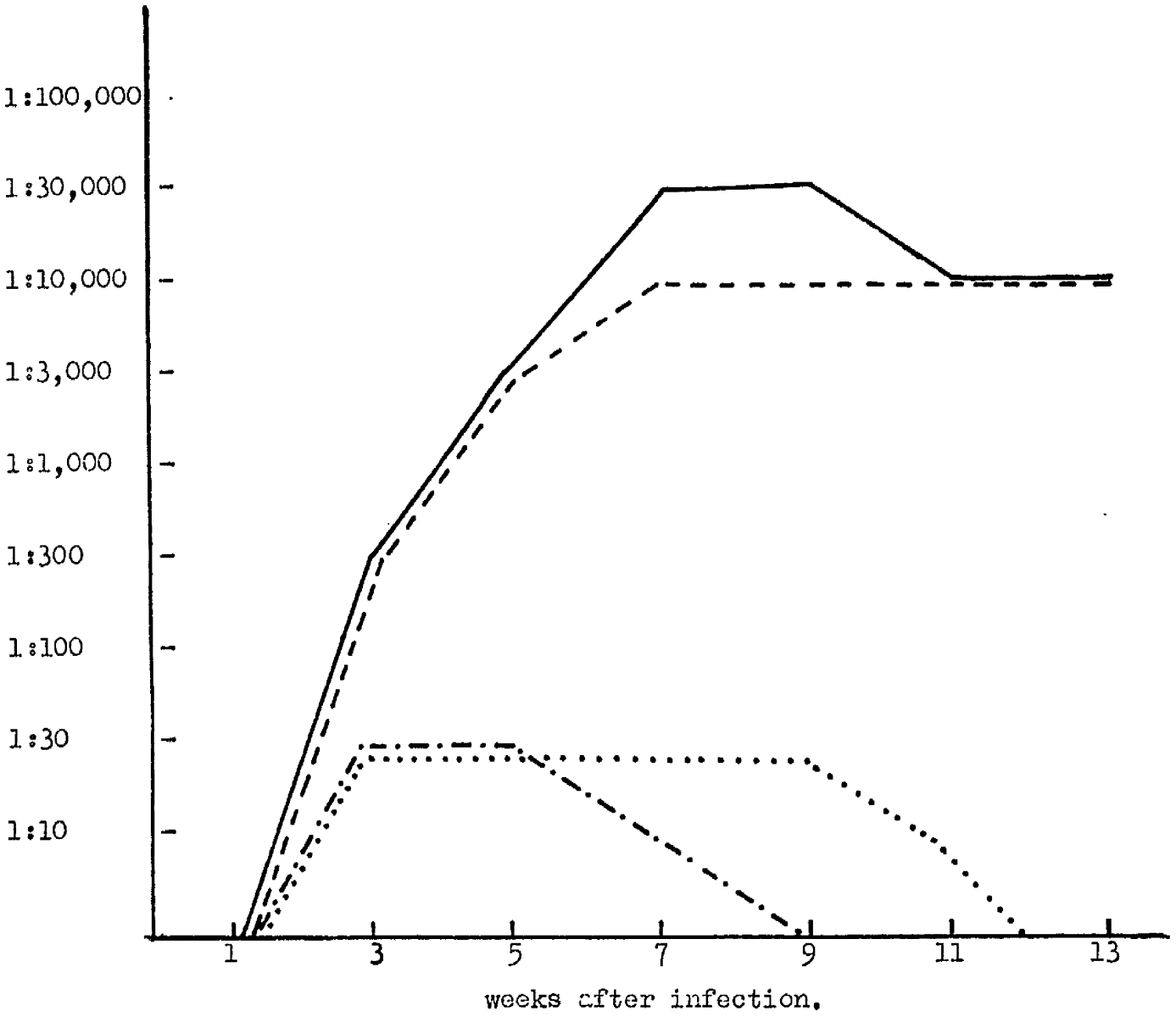


FIG.27. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.18 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —.—.—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

appeared./

Leptospiruria was not demonstrable and abnormalities were not found at post-mortem examination.

Lepto. canicola was recovered from the blood on the fourth day after infection and later was isolated only from renal tissue.

Piglet No. 18.

This animal was obtained from a different litter and was only three weeks old when it was subjected to experiment. Antibodies were demonstrable 21 days after infection to a titre of 1:300 in respect of both strains of Lepto. canicola. With Lepto. icterohaemorrhagiae and with Lepto. pomona cross-reactions to a dilution of 1:30 also occurred. During the next two weeks, Lepto. canicola antibodies increased steadily to a titre of 1:3,000. At the end of the seventh week a reaction with the Aldgate strain occurred to a dilution of 1:10,000, whilst that with strain No. 35667 reached a level of 1:30,000, at which figure it persisted for a fortnight ere it fell to 1:10,000. Thereafter the same titre to both strains persisted throughout the experiment. Cross-reaction with Lepto. icterohaemorrhagiae declined to 1:10 after seven weeks when it disappeared altogether, but that with Lepto. pomona persisted until it fell to 1:10 at the end of the eleventh week and was absent at the time of the final bleeding. Neither with Lepto.

Lepto./

grippotyphosa nor Lepto. hyos was there any reaction. The antibody response is shown in Fig. 27 and the final titres in Table 35.

At the time of infection the body temperature was 103°F. and attained 104.8°F. on the ninth day. A second rise to 104.4°F. took place in the third week and still another increase to 104.5°F. was recorded during the fifth week. Slight pyrexia was noted also during the eighth, tenth and twelfth weeks of the experiment (Fig. 25). On the seventh day after infection, a slight rash was manifest but vanished six days later. At the same time diarrhoea, accompanied by loss of appetite and depression, obtained for about four days. The demonstration of leptospirae in the urine was not successful and post-mortem examination proved negative.

Lepto. canicola was recovered from the blood four days after infection and, post-mortem, from renal tissue alone.

Piglet No. 19.

This piglet came from the same litter as preceding animal and was of the same age, namely, three weeks. As in the previous case, leptospiral antibodies were not demonstrable

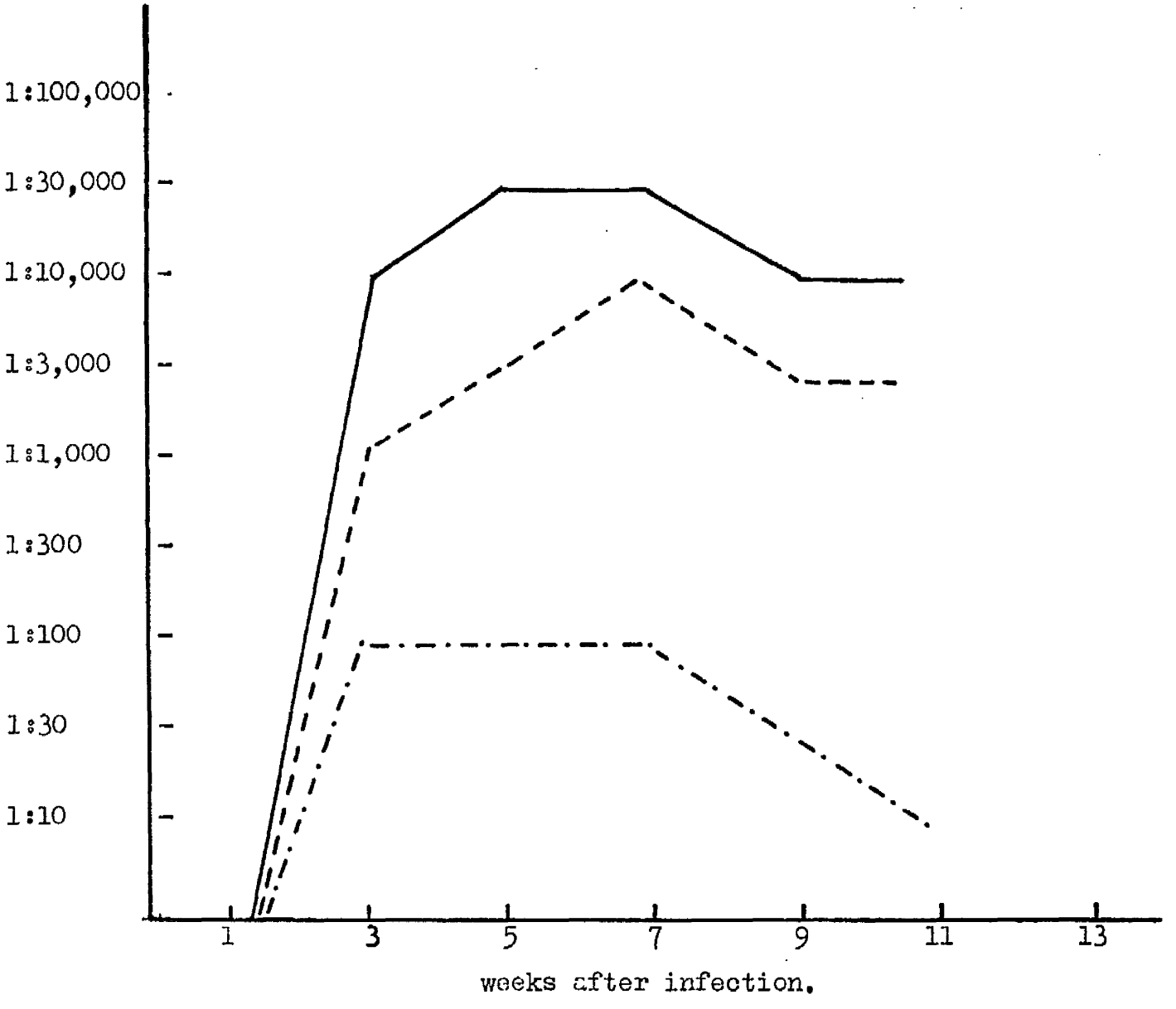


FIG.28. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.19 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

demonstrable/

until the third week after infection, when they occurred to a dilution of 1:10,000 with strain No. 35667 and to 1:1000 with the Aldgate strain of Lepto. canicola. A further increase to 1:30,000 and to 1:3,000 with the corresponding antigens was observed during the following fortnight. At the end of seven weeks, the reaction to the homologous strain had not altered, but the titre to the heterologous antigen had increased to 1:10,000. During the next two weeks the titres fell to 1:10,000 and 1:3,000, respectively, at which levels they remained until the animal's death. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:100 was manifest from the third to the seventh week of infection but had fallent to 1:10 a fortnight later. Cross-reaction with the other antigens did not occur (Fig. 28). The final titres of antibody are presented in Table 35. The temperature chart (Fig. 25) was characterized by a rise of temperature to 104.6°F. which occurred on the ninth day of infection, and by later incidents of moderate pyrexia that happened during the third, sixth and seventh weeks. The pinnacle of fever coincided with the development of a cutaneous rash, which latter lasted for six days. Dullness together with transient diarrhoea were manifest towards the end of the first week of infection. Microscopical examination of samples of urine

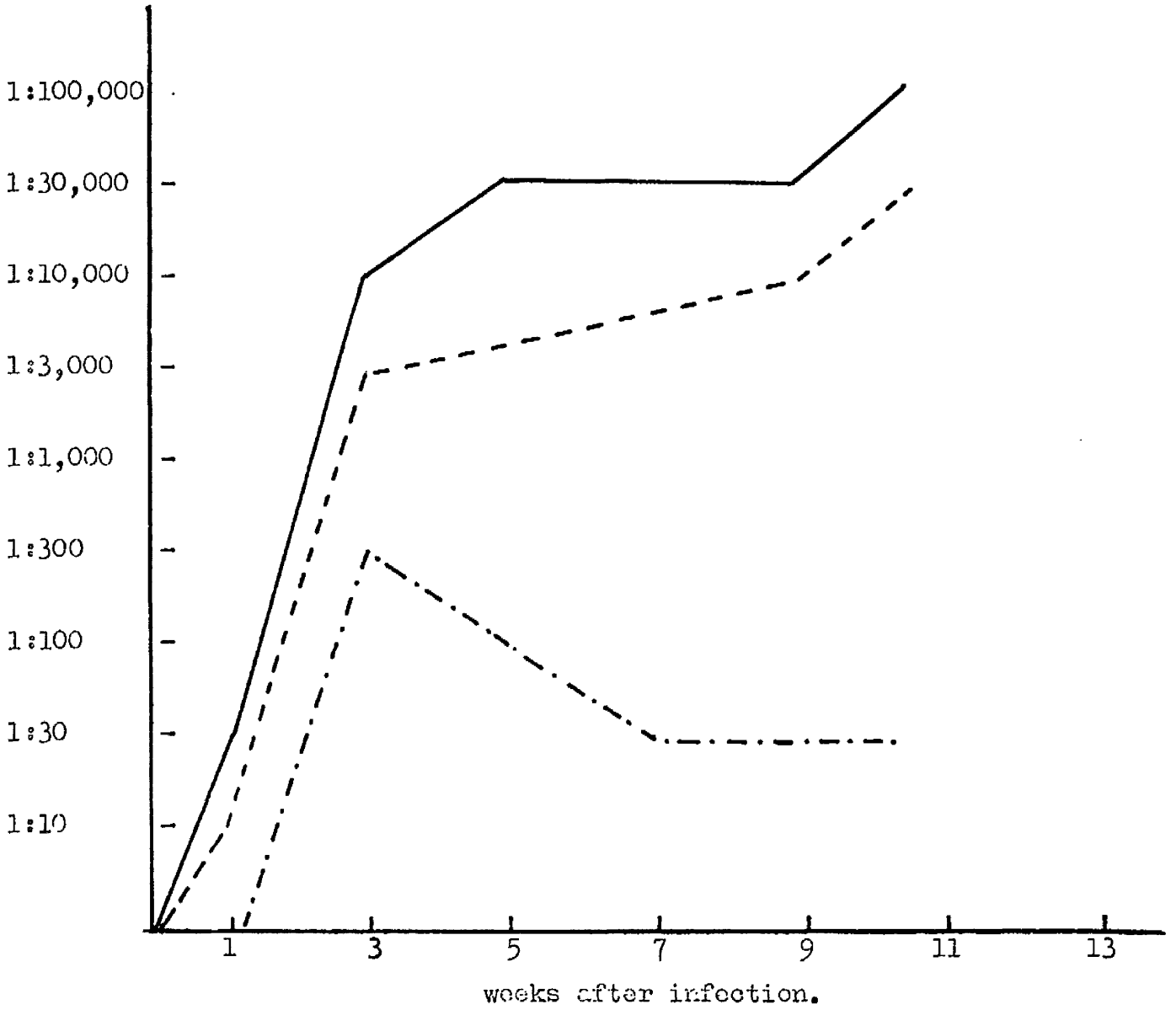


FIG. 29. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No.20 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

urine/

failed to reveal the presence of leptospirae and macroscopic lesions were not observed at post-mortem examination.

All blood cultures made on the fourth day after infection proved positive and leptospirae were later recovered only from the kidneys.

Piglet No. 20.

The titres of antibody in the serum from the first bleeding were 1:30 to the homologous, and 1:10 to the heterologous, strains of Lepto. canicola. Within the next fortnight the titres increased to 1:10,000 and 1:3,000, respectively. At five weeks the titre to strain No. 35667 was 1:30,000 and that to the Aldgate strain was 1:10,000, which levels of antibody persisted until the ninth week of the experiment and, at the time of slaughter one week later, had risen to 1:100,000 and 1:30,000, respectively. Cross-reaction with Lepto. icterohaemorrhagiae to a dilution of 1:300 was established at the end of the third week of infection but had fallen to 1:100 two weeks later and finally to 1:30. A reaction with the other three leptospiral antigens did not occur. The immunological response of this host is presented in Fig. 29 and the final titres are shown in Table 35.

A bodily temperature of 102°F. at the time of

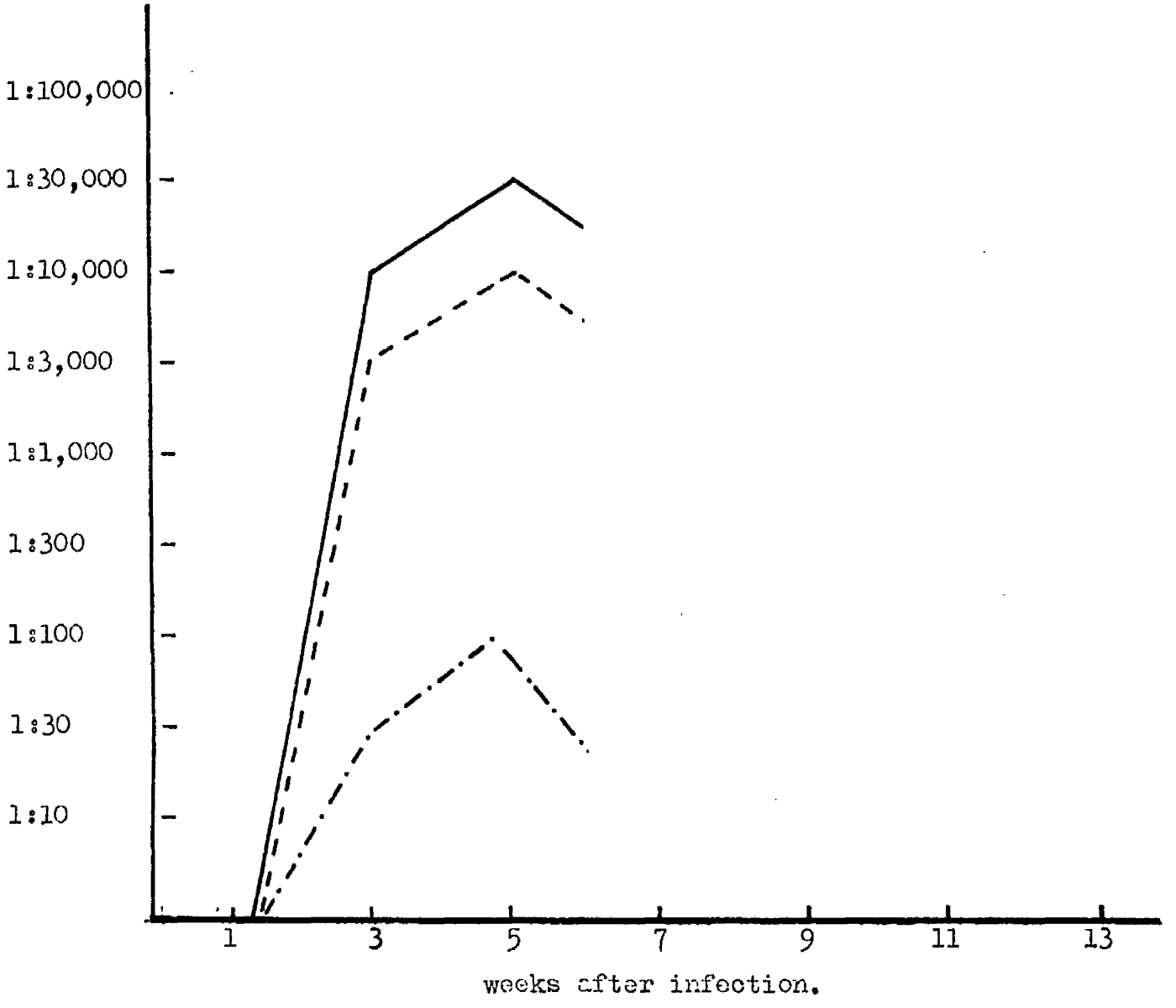


FIG. 30. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 21 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

of/

infection had risen sharply to 105.4°F. on the fifth day but was again normal two days later. Mild fever occurred during the second, fourth, fifth, sixth, seventh and ninth weeks of the experiment (Fig. 25).

Scarcely perceptible cutaneous lesions were manifest from the seventh to eleventh day. Leptospiruria did not occur and gross lesions were not observed at post-mortem examination.

Lepto. canicola was recovered from the blood four days after infection and, post-mortem, only from renal tissue.

Piglet No. 21.

Leptospiral antibodies were not found in the sample of serum taken one week after infection but were detectable at the end of three weeks to a titre of 1:10,000 with strain No. 35667 and of 1:3,000 with the Aldgate strain. After five weeks the respective titres were 1:30,000 and 1:10,000 but had declined to 1:10,000 and 1:3,000 when the animal was killed a week later. Cross-reaction with Lepto. icterohaemorrhagiae was demonstrable from the third week onwards, reaching a maximum of 1:100 at the end of the fifth week but was down to 1: 10 at the time of slaughter. Reaction with the other antigens did not take place (Fig. 30 and Table 35).

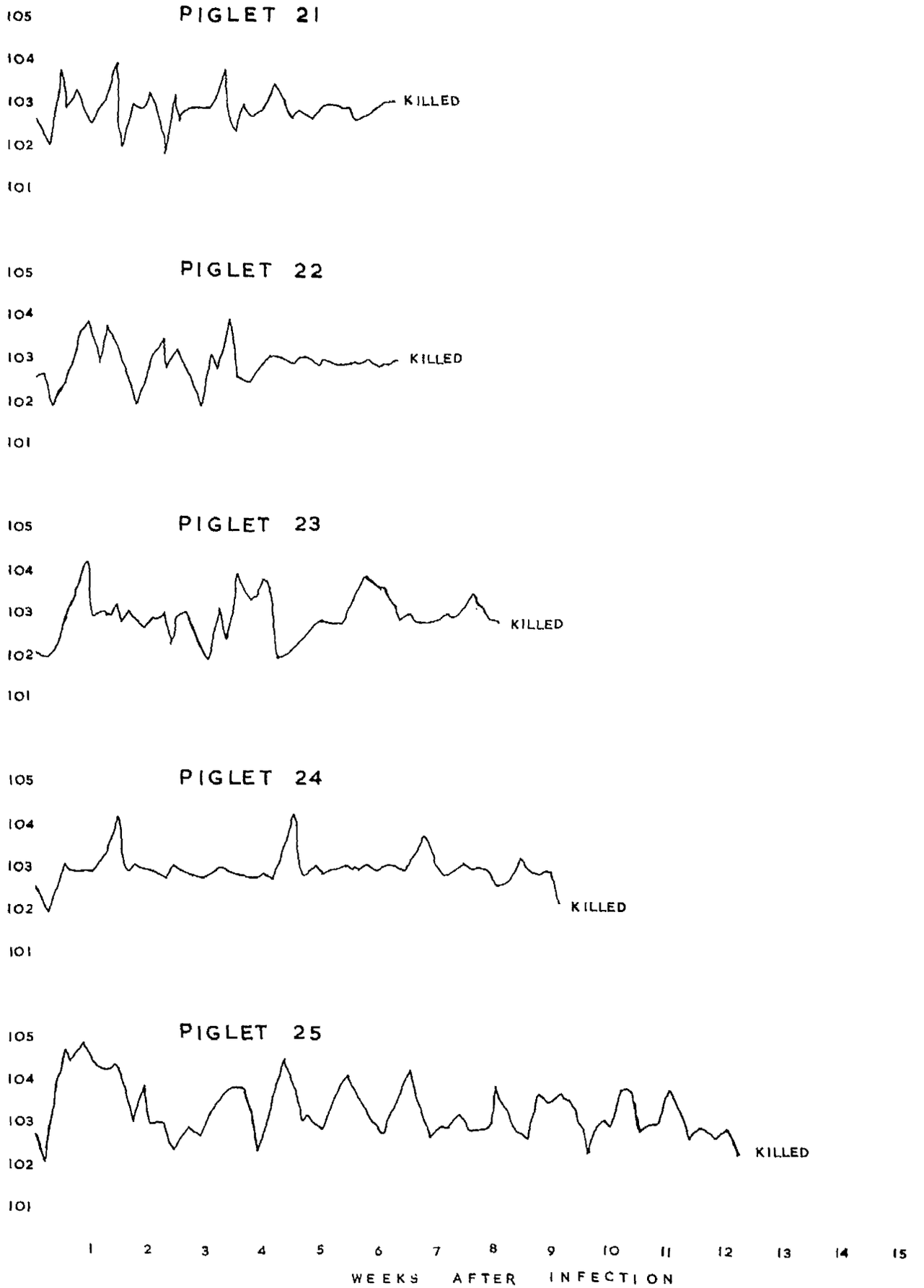


FIG. 31. TEMPERATURE CHARTS OF THE PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

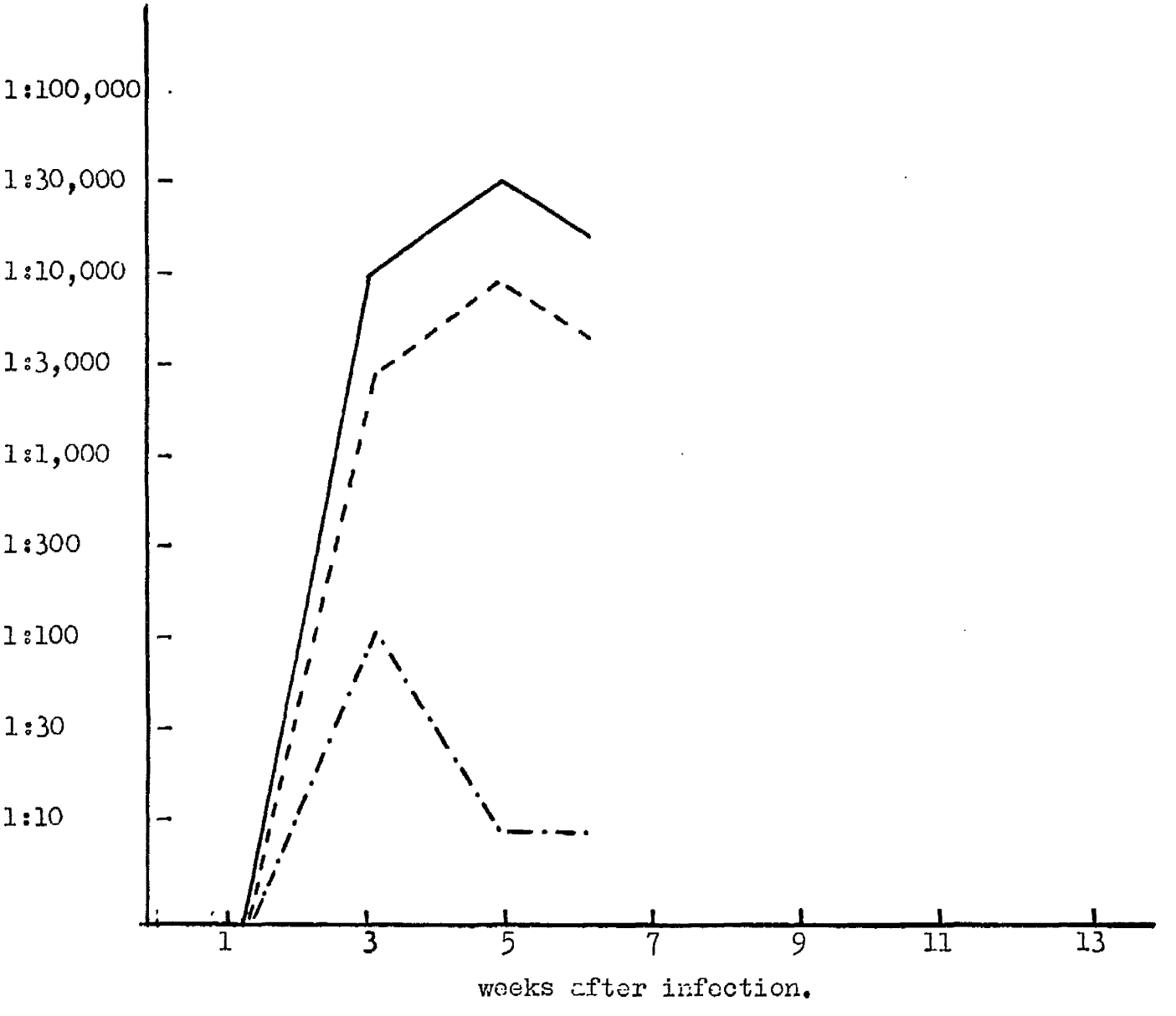


FIG. 32. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 22 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

(Fig. 30 and Table 35)./

As indicated in Fig. 31 a thermal reaction of moderate degree occurred during the first four weeks of infection and, clinically, the animal remained normal throughout the experimental period. Leptospiruria did not supervene and lesions were not found at autopsy.

Lepto. canicola was cultivated from the blood on the fourth day of infection and, after death, was recovered only from the renal tissue.

The kidneys of this animal were stored at 0-4°C. After three days they were found to yield Lepto. canicola in all cultures but the organism grew in only one out of six cultures made after six days of storage.

Piglet No. 22.

Fig. 32 illustrates that the development of leptospiral antibodies was delayed for, at least, one week. At the end of the third week, agglutination-lysis was demonstrable to a titre of 1:10,000 in the case of the homologous type, and to 1:3,000 with the Aldgate strain, of Lepto. canicola. During the following two weeks titres of 1:30,000 and 1:10,000, respectively, were obtained but by the end of the sixth week the amount of antibodies to Lepto. canicola was found to have returned to the level that was demonstrated after the second bleeding.

bleeding./

Cross-reaction was experienced only with Lepto. icterohaemorrhagiae and occurred to a dilution of 1:100 at the end of the third week of infection but had fallen to 1:10 at five weeks. At the time of infection the temperature of the body was 102.7°F. Mild pyrexia occurred six days later and subsequently during the second and the fourth weeks of experiment. Apart from a slight rash, noticeable on the eighth day after infection and during the ensuing three days, the animal did not show any clinical abnormality. Microscopical examination of samples of urine for the presence of leptospirae proved negative and at post-mortem examination macroscopical lesions of the internal organs were not observed. All cultures made from the blood on the fourth day after infection yielded Lepto. canicola.

The organism was later recovered from the kidneys not only immediately after the animal's death but also after the renal tissue had been chilled for a period of six days. Leptospirae were not isolated from any other organs of the body.

Piglet No. 23.

Only a small amount of leptospiral antibody was

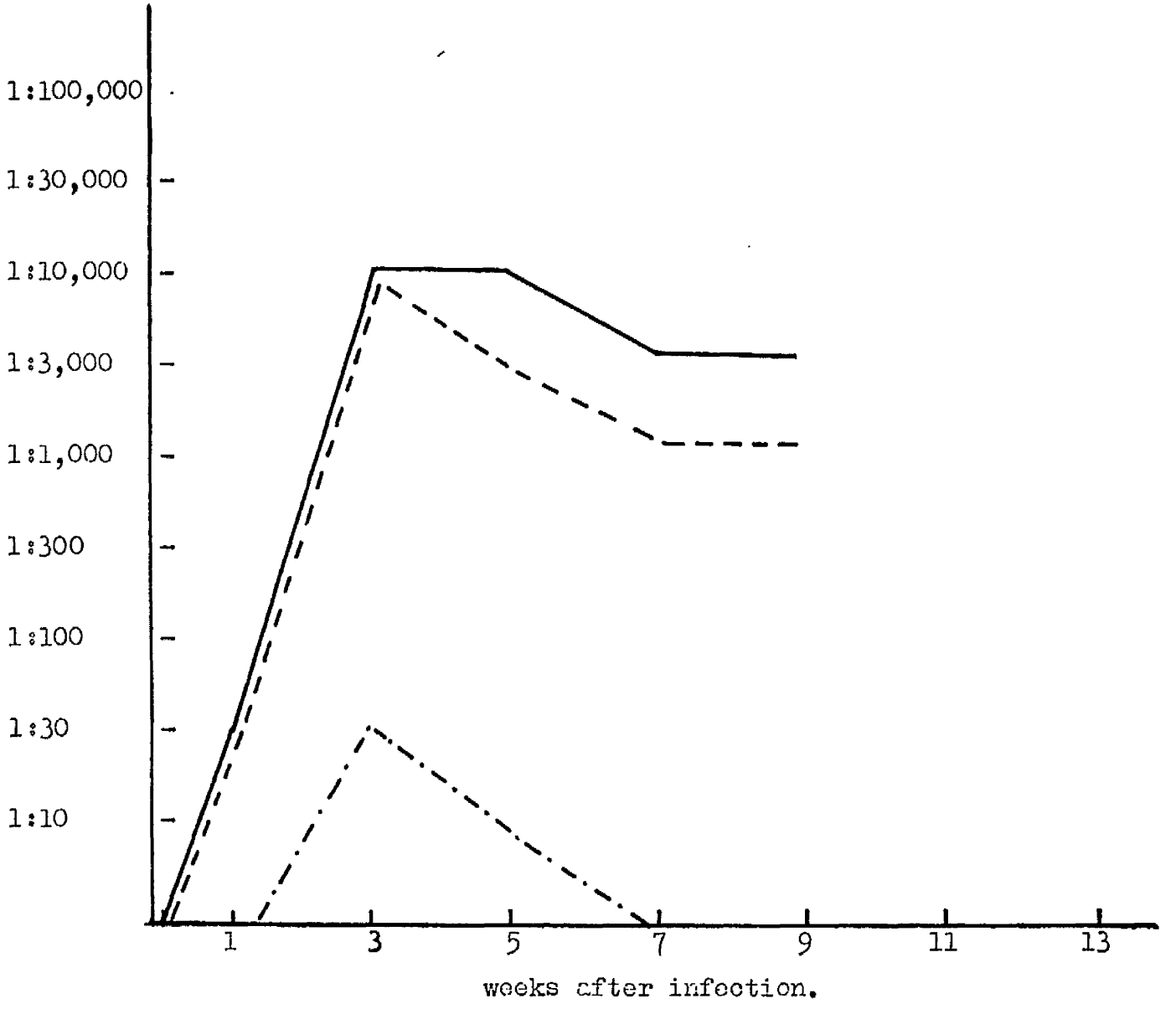


FIG. 33. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 23 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

was/

found one week after infection, when both strains of Lepto. canicola were lysed at a dilution of 1:30. During the next 14 days both titres rose to 1:10,000, which level was maintained for another two weeks in respect of strain No. 35667, but had declined to 1:3,000 with the Aldgate strain. At seven weeks Lepto. canicola antibodies were 1:3,000 (homologous) and 1:1000 (heterologous) and remained so until the end of the experiment. Cross-reaction with Lepto. icterohaemorrhagiae was demonstrable to a dilution of 1:30 at the end of three weeks and to 1:10 at five weeks. Cross-reaction with the other antigens did not occur. The antibody response and the final titres are presented in Fig. 33 and Table 35, respectively. Fig. 31 denotes that a thermal reaction took place at the end of the first week when the temperature rose to 104.4°F. Milder pyrexia was recorded again during the fourth and the sixth week of the experiment. The only clinical feature was a cutaneous rash that appeared on the fifth day after infection and lasted for six days. Autopsical examination proved negative. Leptospirae were detected in a sample of urine that was collected immediately after the animal died.

Lepto. canicola was obtained from the blood on the fourth day of infection. After death, the organism was recovered only from the kidneys and again from renal tissue that

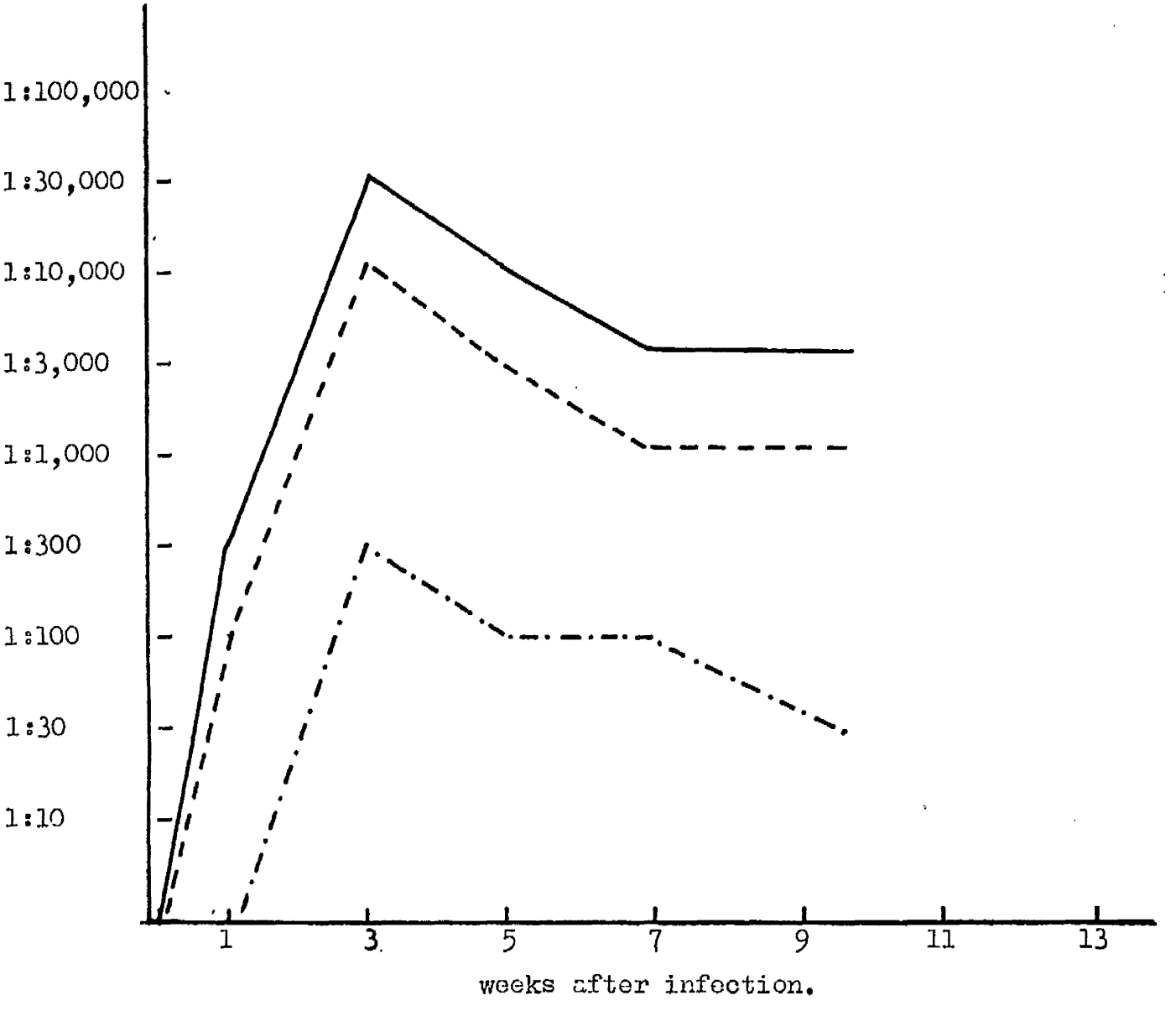


FIG. 34. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 24 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

that/

had been chilled for five days.

Piglet No. 24.

Titres of 1:300 to strain No. 35667 and of 1:100 to the Aldgate strain of Lepto. canicola were demonstrated in the sample of serum obtained at the first bleeding. The respective amounts of antibody rose to 1:30,000 and L;10,000 during the next two weeks, whereupon a steady fall took place so that at five weeks the corresponding titres were 1:10,000 and 1:3,000 and at seven weeks were 1:3,000 and 1:1000. The latter level of Lepto. canicola antibodies obtained when the animal was destroyed at the end of nine weeks. Cross-reaction with Lepto. icterohaemorrhagiae was demonstrable to a dilution of 1:300 at the third week of infection but was manifest to only 1:30 at the close of the experimental period. The graph of antibody titres is shown in Fig. 34.

The final titres and the absence of antibodies to any of the other leptospiral serotypes are indicated in Table 35. The body temperature reached 104.2°F. in the second, and 104.4°F. in the fourth week after infection and excepting a further elevation to 103.8°F. that was recorded during the seventh week of infection, remained within the normal range

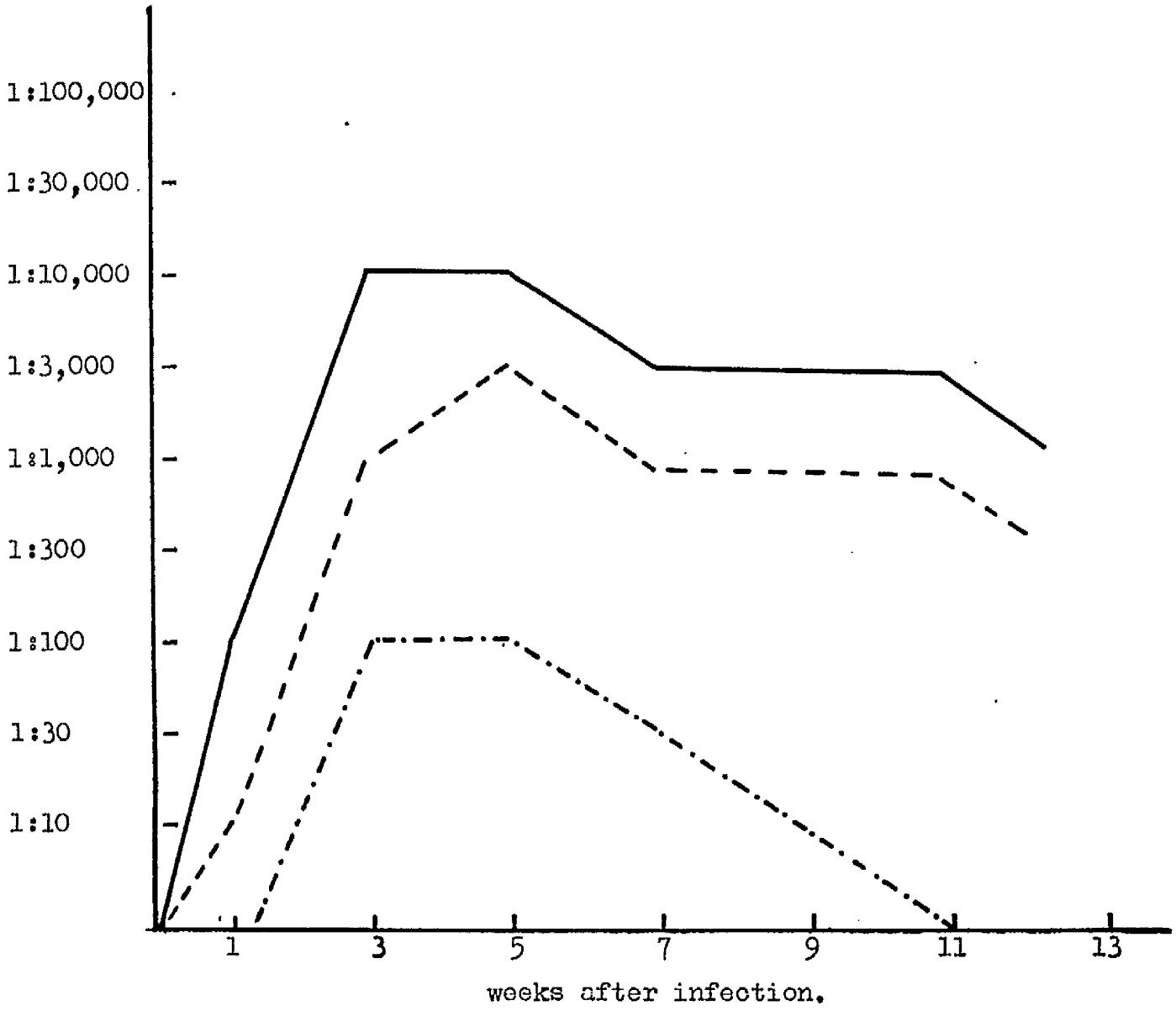


FIG. 35. TITRES OF ANTIBODY IN THE SERUM OF PIGLET No. 25 EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667

Lepto. canicola, Strain No. 35667 ——— Lepto. icterohaemorrhagiae —·—·—
Lepto. canicola, Aldgate Strain — — — Lepto. pomona .....

range/

(Fig. 31).

One week after infection there was manifest on the skin of the abdomen and of the insides of the thighs a slight rash that vanished seven days later. Leptospirae were found to be present in the urine eight weeks after infection. Postmortem, gross lesions were not observed and Lepto. canicola was recovered from the renal tissue (as, indeed, the organism had been obtained from the blood four days after infection).

Piglet No. 25.

At first, the antibody response was feeble inasmuch as, one week after infection, the titre to strain No. 35667 did not exceed 1:100 whilst that to the heterologous strain was only 1:10. During the next fortnight, the titre to the homologous strain rose to 1:10,000, at which level it remained for another two weeks ere it fell to 1:3,000 at seven weeks and finally to 1:1000 at twelve weeks. The reaction to the heterologous strain was 1:1000 at the third week of infection, 1:3,000 two weeks later but declined to 1:1000 at the end of the seventh week and was 1:300 at the close of the experiment. From the third to the fifth week, cross-reaction with Lepto. icterohaemorrhagiae occurred to a dilution of 1:100 but declined to 1:10 four weeks later and was not demonstrable with the samples of serum obtained

obtained/

at the last two bleedings. Reactions to Lepto. crippotyphosa, Lepto. hyos or Lepto. pomona were not forthcoming (Fig. 35). The final titres are summarized in Table 35. The temperature of the body rose to 105°F. on the fifth day and remained at 104.4°F. for the ensuing four days. Pyrexia of 103.6°F. to 104.4°F. was recorded during the fourth, fifth, sixth and seventh week and the temperature rose again to 104.5°F. in the ninth week (Fig. 31). Once again, a slight rash was the only clinical finding. Leptospiruria was demonstrable at the time of slaughter, that is, at twelve weeks after infection. Gross lesions were not visible at post-mortem examination. Lepto. canicola was isolated from the blood four days after infection and, after death, was obtained only from the kidneys.

Piglet No. 26 (Control).

Table 35 indicates that throughout the period of the experiment the serum of this control animal remained free from all leptospiral antibodies. The temperature of the body lay between 101.4°F. and 103°F. (Fig. 36). The animal remained in good health, leptospiruria did not occur, lesions of the internal organs were not manifest at autopsy and all cultures proved negative.

TABLE 35.

TITRES OF ANTIBODY IN THE SERA OF PIGLETS EXPERIMENTALLY INFECTED BY LEPTO. CANICOLA, STRAIN No. 35667.

Piglet No.	Route of infection	L. canicola strains: No. 35667	L. canicola strains: Alagate	Lepto. ictero-h.	Lepto. pomona	Lepto. grippo-t.	Lepto. hyos.
1	s/c.	1:30,000	1:10,000	1:10	-	-	-
2	"	1:10,000	1:3,000	1:30	-	-	-
3	"	1:30,000	1:10,000	1:10	1:30	-	-
4	skin scarific.	1:10,000	1:1000	1:100	-	-	-
5	"	1:10,000	1:3,000	1:30	-	-	-
6	"	1:30,000	1:10,000	1:10	-	-	-
7	Nil(C)	-	-	-	-	-	-
8	skin scarific.	1:1000	1:1000	-	-	-	-
9	"	1:10,000	1:3,000	1:10	-	-	-
10	"	1:10,000	1:3,000	1:10	1:30	-	-
11	"	1:1000	1:300	-	-	-	-
12	"	1:10,000	1:3,000	1:10	-	-	-
13	"	1:1000	1:1000	-	-	-	-
14	Nil(C)	-	-	-	-	-	-
15	s/c.	1:30,000	1:3,000	1:30	-	-	-
16	"	1:100,000	1:30,000	1:100	1:100	-	-
17	"	1:30,000	1:10,000	1:300	-	-	-
18	"	1:30,000	1:10,000	1:30	1:30	-	-
19	"	1:30,000	1:10,000	1:100	-	-	-
20	"	1:100,000	1:30,000	1:300	-	-	-
21	"	1:30,000	1:10,000	1:100	-	-	-
22	"	1:30,000	1:10,000	1:100	-	-	-
23	"	1:10,000	1:10,000	1:30	-	-	-
24	"	1:30,000	1:10,000	1:300	-	-	-
25	"	1:10,000	1:3,000	1:100	-	-	-
26	Nil(C)	-	-	-	-	-	-

(C) = Control Animal.

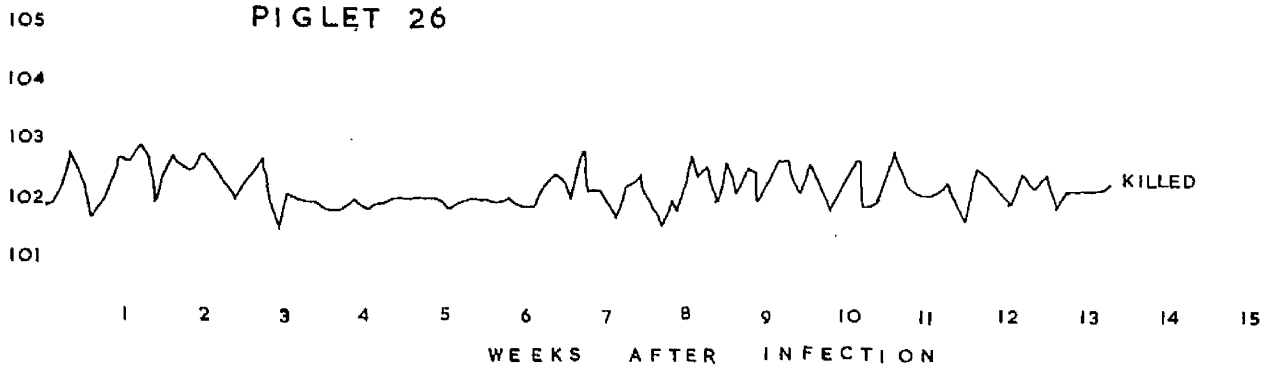


FIG. 36. TEMPERATURE CHART OF THE CONTROL PIGLET No. 26.

negative./

4. EXPERIMENTAL ORAL INFECTION OF A YOUNG DOG.

That raw kidneys from carrier animals may constitute a source of infection for dogs (Michna, 1959a) is supported by an experiment in which a six weeks-old collie puppy, that did not have any leptospiral antibodies in its serum, was fed two pieces of raw pig kidney, each of about 10 grams in weight, over which a culture of Lepto. canicola, Strain No. 35667, had been newly sprinkled. The culture had been under artificial cultivation for only four weeks. Three weeks later the pup's serum was found to contain Lepto. canicola antibodies to a dilution of 1:300. After a further three weeks the titre had risen to 1:10,000 and four weeks later still had reached a maximal level of 1:100,000.

At the latter date, too, a titre of 1:30 to Lepto. icterohaemorrhagiae was also manifest. Frank leptospiruria was detectable one month after infection and continued for twelve weeks at the end of which period only a few non-motile leptospirae were observable. Coincident with the waning leptospiruria was the finding of antibodies to Lepto. canicola and Lepto. icterohaemorrhagiae to dilutions of 1:300 and 1:10, respectively. By this time, the puppy was obviously ill and manifested marked depression, anorexia, vomiting, thirst and

and/

loss of bodily condition. On the two occasions on which it was recorded, fever of 104.5°F. and 105°F. obtained. There was moderate vascular congestion of the conjunctivae accompanied by a sero-mucoid discharge. Estimations of the level of blood urea, carried out in the Department of Veterinary Biochemistry, according to the method of King (1951), yielded the following results in mg. per 100 ml.:

52.0 at six weeks after infection,
95.0 at nine do. do.
75.5 at fourteen do. do. and
49.0 at eighteen do. do.

Recovery was gradual and eventless.

5. DISCUSSION.

Coghlan et al. (1957) were unable to detect the presence of specific antibodies in one out of five piglets that had been artificially infected by Lepto. canicola. Herbert-Burns and Flavell (1951) and Marcuse and Pohlman (1952), quoted by Alston and Broom (1958), suggested that antibodies may be absent in some cases of leptospirosis, a view that Alston and Broom did not share. In the present series of experiments, not only was the infective dose made to vary in size but the route

route/

of administration also was diversified. Nevertheless antibodies became demonstrable in the sera of all the infected animals, although the times of their appearance were found to differ. In 11 out of 23 piglets (47.8 per cent.), antibodies occurred in concentrations of from 1:30 to 1:3,000, one week after infection but in the case of the remaining 12 sera antibodies appeared in from two to three week's time to titres ranging from 1:100 to 1:10,000. Such a finding accords with the statement of Broom (1959). In six out of nine piglets (66.6 per cent.), infected by skin scarification, there was a delay of from one to two weeks ere specific antibodies appeared, whereas in nine out of fourteen piglets (64.4 per cent.) infected by the subcutaneous route, leptospiral antibodies were demonstrable at the end of the first week of experiment. In three of the piglets, the highest titre ever recorded was 1:1000 but in two other animals antibody to the homologous strain of Lepto. canicola was demonstrable to a dilution of 1:100,000. The remaining eighteen animals presented titres that varied from 1:10,000 to 1:30,000. Excepting two piglets which were killed after a fortnight, maximal production of leptospiral antibodies occurred: in 2 cases (8.7 per cent.) after two weeks of infection, in 10 cases (43.5 per cent.) do. three do. do.

in 10 cases (43.5 per cent.) after three weeks of infection./

in 1 case (4.3 per cent.) do. four do. do.

in 3 cases (13.0 per cent.) do. five do. do.

in 1 case (4.3 per cent.) do. six do. do.

in 2 cases (8.7 per cent.) do. seven do. do.

in 2 cases (8.7 per cent.) do. ten do. do.

In all but one piglet, in which the titre was 1:100,000 at the time of slaughter, the amount of antibody declined slightly after the peak of development, but still persisted to high dilutions until the end of the experiment, that is, for a period of from two weeks to three and a half months. Lovell (1943) was of the opinion that in leptospirosis "agglutinins, lysins and complement-fixing antibodies appear towards the end of the first week of disease, increasing to a high level within 5-8 weeks. They persist for many months and then decline over a period of one to three years". Further in respect of antibodies, Broom (1959) said: "They reach a maximum about the fourth week and then slowly decrease in amount over months or years".

The antibody response of the host may be influenced by certain factors, such as: the size of the infective dose, the virulence of the invading leptospira, the portal of entry, the age, and above all the individuality of the animal.

animal./

The first group of experiments was carried through with Strain No. 35667 of Lepto. canicola which had been maintained under artificial conditions for a period of 14 months after its isolation from natural sources. The animals of groups 2 and 3 were infected by a second or third subculture of the same strain after it had been recovered from some of the subjects used in the first group. In so far as the creatures in groups 2 and 3 are concerned, it is reasonable to assume that leptospiral virulence was unlikely to have differed substantially. Although two different modes of infection were employed, identical antibody responses were not forthcoming, as is shown by the various graphs. Generally, the titres to Strain No. 35667 were higher than those to the Aldgate one. Nearly all of the piglets gave evidence of cross-reaction with Lepto. icterohaemorrhagiae, which is a not unusual phenomenon.

In the case of four piglets, cross-reaction with Lepto. pomona was also demonstrated. In the latter connection, Lang and Morse (1959) studied cross-reactions given by the sera of different animal species infected by Lepto. pomona and found that they consistently occurred with Lepto. icterohaemorrhagiae and Lepto. canicola antigens in the case of sera derived from infected sheep, goats and pigs. Guinea-pig sera cross-reacted

cross-reacted/

with Lepto. canicola, but only with the A strain of Lepto. icterohaemorrhagiae.

A three-to seven-fold increase of the total white cell count, with a rise of the neutrophils of up to 7 per cent. above the pre-infection level, is an index of the individual response of the host. A search of the available literature has failed to reveal any report on the haematological examination of pigs infected, either naturally or experimentally, by Lepto. canicola. Schmidt and Giovanella (1947), however, found percentages of 57.5 of neutrophils, 35 of lymphocytes, 2.5 of monocytes and 2.5 of eosinophils in the blood of a piglet five days after the creature had been infected by Lepto. pomona. In another similarly infected piglet, a blood count, at three months after infection, showed percentages of 32.5 of neutrophils, 56.5 of lymphocytes, 5.0 of monocytes and 6.0 of eosinophils. On the other hand, during a study of the pathology of Lepto. pomona infection of swine, Morse et al. (1958) did not find any alteration of either the cell counts, the level of haemoglobin or the amount of non-protein nitrogen in the plasma. The thermal reactions were far from uniform. The body temperature of three piglets did not differ from that of the control animals but in 15 cases it rose to 104-104.8°F, while in five other instances it increased to

to/

105°F. or slightly over. An undulant type of fever was observed in some of the animals. Bezdenezhmykh and Kashanova (1956) encountered a temperature of 41°C. (105.8°F.), that lasted for 3-5 days, during an outbreak of Lepto. canicola infection of piglets which occurred on collective farms on the Russian Island of Sakhaline. In the course of the experiments carried out by Coghlan and her co-workers (1957), significant change of body temperature and clinical signs did not follow experimental inoculation of a culture of Lepto. canicola but, in the case of two piglets infected by urine containing leptospirae, fever of 106°F. and of 104.6°F. was recorded on the sixth and on the tenth days, respectively.

The occurrence of cutaneous changes in pigs infected by Lepto. canicola does not appear yet to have been recorded but such lesions were observed in all but one of my experimental animals. In canicola fever of man, however, erythema may appear in about 19 per cent. of cases. Indeed, Alston and Broom (1958) have written "Rashes, either morbilliform, scarlatiniform or herpetic in type, or, later, in the illness, a rash resembling the rose spots of typhoid fever frequently appear, but these eruptions are often fleeting and may not be noticed unless they are specially looked for".

for"./

Gayot (1955), too, described cutaneous ulcers in cattle that suffered from acute infection by Lepto. grippotyphosa. In general, porcine infection due to Lepto. canicola is lacking in clinical signs and has seldom been considered as of much economic importance. Nevertheless, recent evidence suggests that the condition may be of some significance to public health.

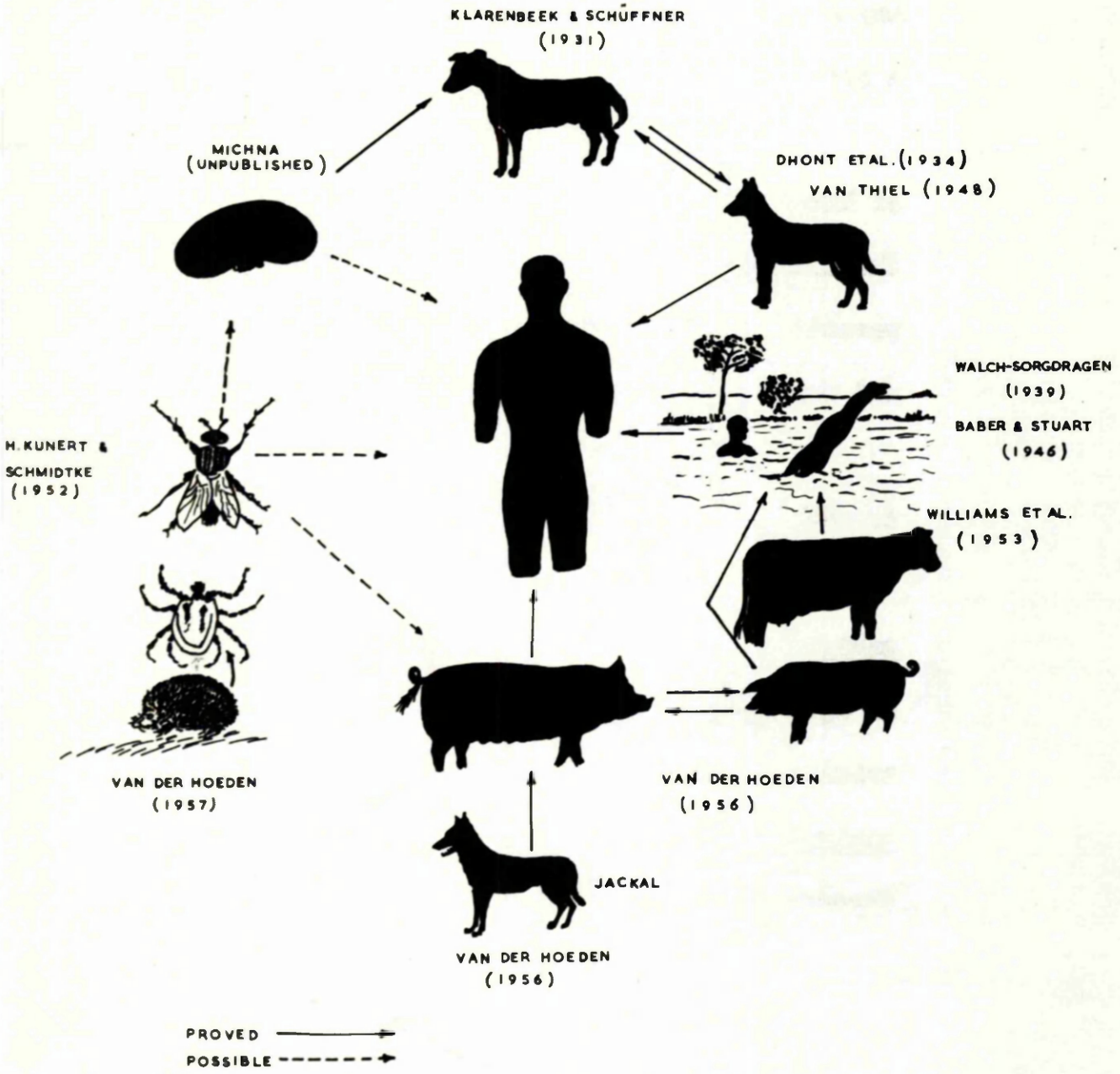
In this series of experiments there were three piglets, of three to five weeks of age, which manifested diarrhoea, loss of appetite and dullness and conjunctivitis occurred in one of those cases. Similar signs together with icterus and haemoglobinuria and associated also with heavy mortality among young piglets were reported by Bezdenezhnykh and Kashanova (1956). It may be, therefore, that clinical abnormality is more likely to be evident in younger animals afflicted by Lepto. canicola. There is little doubt also that poor hygiene, environmental conditions and level of nutrition are important contributory factors in cases of natural disease. Since macroscopic lesions not always develop in the internal organs, the naturally infected carcasses are unlikely to be detained by meat inspectors. In three out of twenty-three animals (13 per cent.), microscopical examination sufficed to reveal the presence of leptospirae in renal tissue. In ten, (43 per cent.), of the

the/

experimental animals, leptospiruria was demonstrated at times varying from the fourth to the twelfth week of infection. In 73.9 per cent. of cases, Lepto. canicola was recovered from the blood at 4-7 days after infection, that is, during the stage of leptospiraemia. Although Lepto. canicola was isolated from the kidneys of 20 out of 23 experimentally infected piglets (87 per cent.), all of those animals yielded serological evidence of infection. Since after the leptospiraemic phase the organism was obtainable only from the kidneys, those organs of the body would appear to constitute the principal site of localization and, whether it is in the fresh state or had been stored at a low temperature for several days, infective renal tissue must be regarded as a not unimportant risk to both man and animals.

The present studies would support the view that "other sources" (McIntyre and Seiler, 1953) of infection may include not only the kidneys of carrier pigs or cattle but also any food or feeding stuff that has been contaminated by urine. Kunert and Schmidtke (1952) demonstrated that leptospirae may live for at least 26 hours in the crop and in the intestine of non-blood-sucking flies. It is not impossible that both stable

Fig. 37. THE CYCLE OF INFECTION OF MAN AND ANIMALS BY LEPTOSPIRA CANICOLA



stable/

and domestic flies may act as vectors of Lepto. canicola and, since it is the habit of those insects to swarm about the eyes, the nostrils and the mouth of dogs and other animals, they may deposit spirochetes at consequential portals of entry. Such a problem is, indeed, worthy investigation.

Fig. 37 is designed to illustrate the present state of knowledge regarding host susceptibility to Lepto. canicola, the animals which may act as carriers or as vectors of the parasite and the possible sources and modes of infection of man and animals.

6. SUMMARY.

1. The antibody response to infection by Lepto. canicola was studied in 26 piglets, from three to eight weeks of age. Three of the animals served as controls and the remaining 23 were artificially infected with a culture of Lepto. canicola, (Strain No. 35667) which was administered either through the scarified skin or by subcutaneous inoculation.

2. All the infected animals developed leptospiral antibodies but the individual responses varied considerably. Titres to the homologous (No. 35667) strain of Lepto. canicola extended from 1:1000 to 1:100,000 while those to the heterologous (Aldgate) strain ranged from 1:300 to 1:30,000.

1:30,000.

3. Cross-reactions with Lepto. icterohaemorrhagiae to dilutions of from 1:10 to 1:300 occurred in the case of twenty piglets.

4. The sera of four infected animals gave also cross-reaction to Lepto. pomona to titres of from 1:30 to 1:100.

5. Reactions with Lepto. grippotyphosa or with Lepto. hyos were not encountered.

6. Throughout the experiment leptospiral antibodies were not found in the serum of any of the control piglets.

7. Haematological examination revealed that the total white cell count of infected animals was increased three- to seven-fold.

8. Five piglets showed an increase of body temperature of up to 105°F. or slightly over, while in fifteen animals there was pyrexia of 104 and 104.8°F. In three cases the temperature did not differ from that of the control piglets.

9. A rash, varying in intensity and in duration, was present in all but one of the infected creatures.

10. In three cases, two of which involved animals only three weeks old, clinical illness of a few day's duration was manifest in diarrhoea, dullness and loss of appetite, and

and/

conjunctivitis also was observed in one instance.

11. Macroscopical lesions were not detected in the internal organs of piglets killed at different stages of the experiment.

12. Leptospirae were demonstrated in wet films made from the supernatant fluid of macerated kidneys taken from three of the infected animals.

13. Leptospiruria was established in the case of ten animals, at times which varied from the fourth to the twelfth week of infection.

14. By means of blood culture, leptospiraemia was shown to exist at four days after infection in the case of fifteen animals and at seven days after infection in three animals.

15. In the case of twenty of the infected animals, which were killed two to fourteen weeks after infection, Lepto. canicola was recovered from the kidneys immediately after slaughter and again from those organs after they had been chilled for twelve days or frozen for six days.

16. Attempts to isolate Lepto. canicola from the liver, spleen, lungs, lymph-nodes and skeletal muscles of infected piglets were not successful.

successful./

7. GENERAL DISCUSSION.

The results of this serological survey tend to substantiate the evidence adduced by previous workers that infection by Lepto. icterohaemorrhagiae may involve pigs in the British Isles. Moreover, the survey gives proof of a much wider incidence of infection by Lepto. canicola in Scottish piggeries and has revealed the possibility of infection by Lepto. pomona, as well. The latter contingency, however, requires investigation.

The isolation of ten cultures of Lepto. canicola from naturally infected Scottish pigs was not only the first recorded experience of its kind but also claimed to be of considerable significance inasmuch as in nine cases the organism was recovered from the kidneys of clinically healthy pigs which had been passed for human consumption. Moreover, the animals concerned originated from three out of five premises in which the presence of infection had been determined by serological means. In one case, the recovery of Lepto. canicola from porcine renal tissue succeeded the receipt of information concerning a case of canicola fever that had been detected in an employee of the piggery.

piggery./

In the sera of all experimentally infected piglets Lepto. canicola antibodies developed within one to three weeks to titres of from 1:1000 to 1:100,000.

Possible sources of infection for man and other animals comprise not only the blood, especially during the leptospiraemic phase, but also the urine voided from, approximately, the third week of infection onwards and the kidneys of slaughtered carrier animals. The problem of Lepto. canicola infection in pigs seems scarcely less important from the point of view of public health since affected animals generally do not show any clinical abnormality and seldom are macroscopic lesions evident at post-mortem examination. Coghlan et al. (1960) have recorded infection by Lepto. canicola that persisted for as long as five years in two piggeries on the outskirts of Edinburgh and established that eleven out of sixteen cases of human canicola fever were traceable to such sources. Meat and other victuals containing leptospirae may be responsible for outbreaks of disease in both man and animals. Alisowa (1946) reported a number of cases of human leptospirosis which followed the consumption of milk that came from cattle previously afflicted by leptospirosis. Lubaszenko (1948) noted that the feeding of infective carcasses to silver foxes culminated in

in/

leptospirosis and that experience led him to assert that the slaughter, for human consumption, of animals affected by acute leptospirosis should be forbidden, especially if jaundice and cachexia be apparent. Cyss (1951) produced leptospirosis in puppies and kittens as the result of the feeding of meat from artificially infected calves and recommended that such meat could be made innocuous if it was treated with a 4.8 per cent. solution of sodium chloride for not less than ten days. Kotowa (1955) studied the survival of leptospirae in the flesh of artificially infected sousliks and lambs as well as their persistence after they had been introduced into pieces of rabbit muscle. The results were rather conflicting for, although virulence was appreciably reduced during the 48 hours necessary for ripening of the meat, in meat dried for eight days the virulence did not alter. On the other hand, a marked loss of virulence occurred in meat that had been dried for thirteen days. In vitro, lactic acid at pH 5.4-5.6 was found to kill leptospirae within 72 hours. Shitow (1955) proved that the meat of creatures, that had suffered from clinical leptospirosis or were in the incubative stage of the disease, may be a source of infection for man and animals. Mochman and Mahnke, (1959) too have emphasized how dangerous for man may be pigs infected by

by/

leptospirae.

Since the completion of this work, three more cases of canicola fever among piggery workers have been brought to my notice. One was in a patient at Strathclyde Hospital, Motherwell, who had been employed in a piggery in Blantyre (personal communication from Dr. R. S. Dewar, 1960). The other two were young men who had been employed on two different pig-farms at Lenzie, near Glasgow, before they were admitted to Ruchill Hospital, with typical meningitic symptoms and in whose blood serum Lepto. canicola antibodies were demonstrated to a dilution of 1:10,000 (personal communications from Dr. J. H. Lawson, 1960 & 1961).

In samples of serum obtained from twenty pigs reared on one of the farms antibodies to Lepto. canicola were found in dilutions ranging from 1:100 to 1:30,000, and the organism was recovered from the kidneys of eight of those animals (culture L_1). In five out of six samples of pig serum procured from the other Lenzie farm antibodies were found to be present to dilutions of from 1:300 to 1:30,000, and the organism was isolated from the kidneys of three of the animals (culture L_2). Some of the pig kidneys originating from both farms contained whitish spots that are illustrated in Figures 38 and 39.

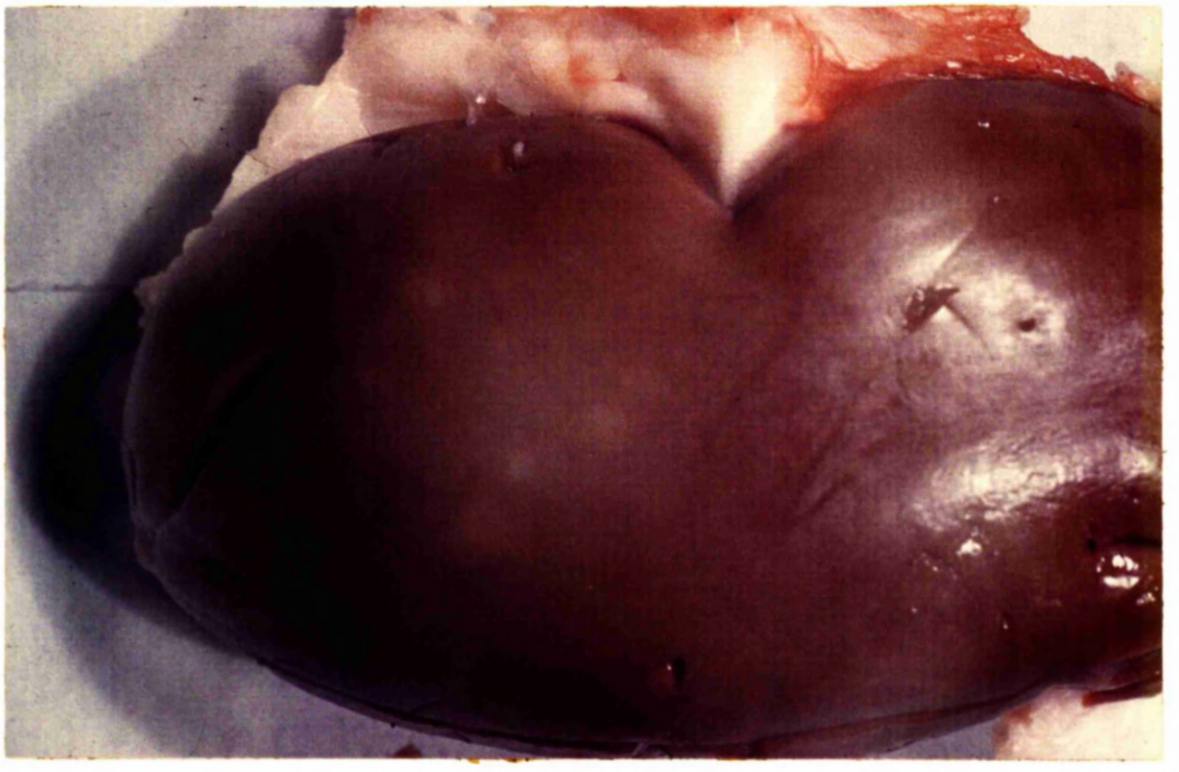


Fig. 38. Whitish foci in the pig kidney from which Lepto. canicola (Strain L_1) was recovered.

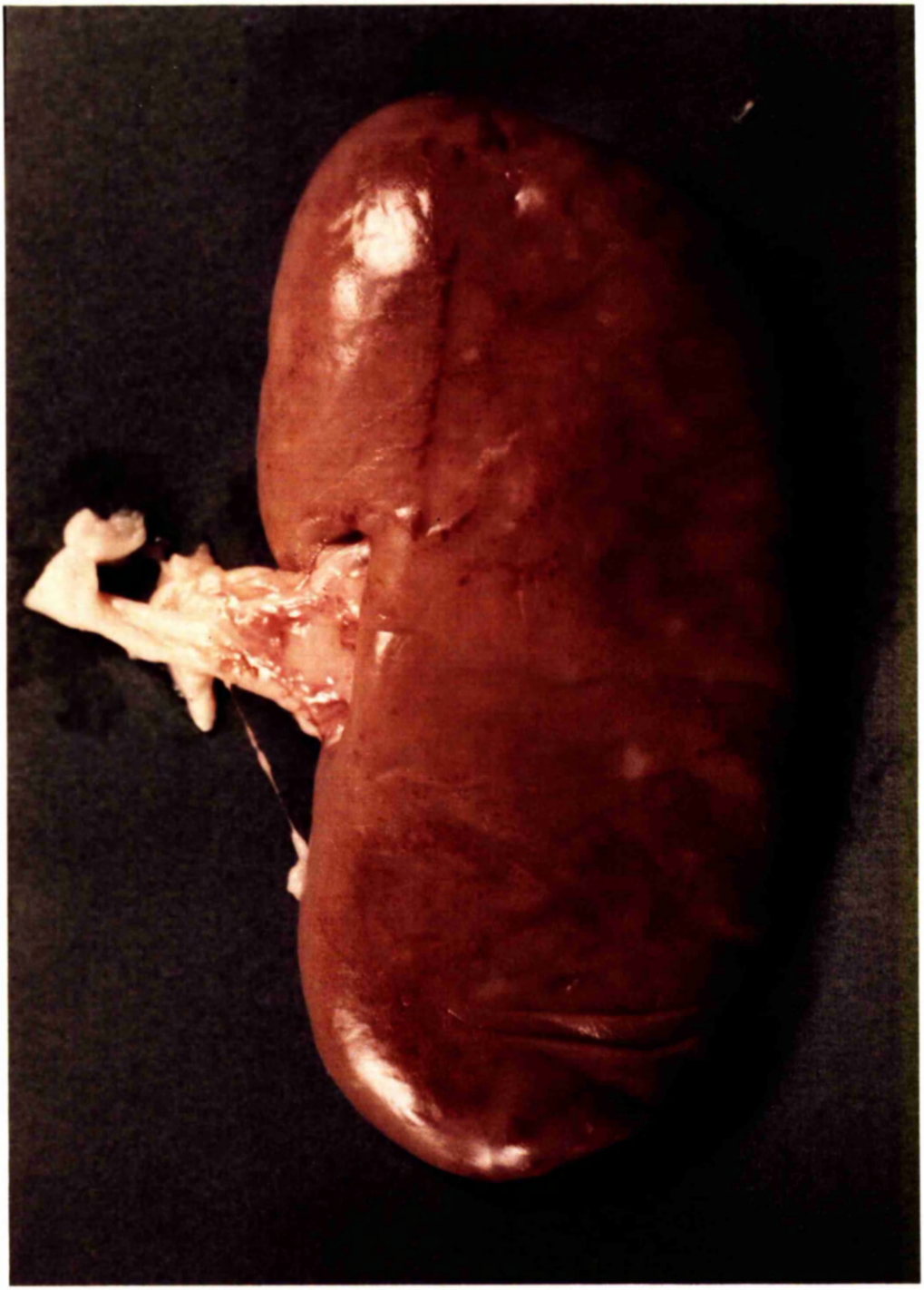


Fig. 39. Numerous yellowish foci and petechial haemorrhages in the pig kidney from which Lepto. canicola (Strain L2) was recovered.

39./

It would appear, therefore, that three more piggeries in the Glasgow area must be added to the list of premises already given in parts 1 and 2 of this thesis, making a total of eight holdings on which infection by Lepto. canicola has been established to exist.

The feeding to a six weeks' old puppy of pieces of raw pig kidney containing Lepto. canicola produced typical leptospirosis canicolaris. It has also been shown that Lepto. canicola may survive in chilled (0-4°C.) porcine kidneys for up to twelve days and for at least six days of storage at -8°C. Thus, the human beings exposed to immediate risk of leptospirosis include not only piggery attendants but also slaughtermen, butchers, meat traders, cooks and housewives. Infection of man by Lepto. canicola, therefore, deserves to be considered as an occupational zoonosis.

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