J. Hyg., Camb. (1962), **60**, 427
Printed in Great Britain

Factors affecting the development of the carrier state in leptospirosis

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(*Received* 1 *June* 1962)

INTRODUCTION

Leptospirosis provides a useful model for the study of the carrier state in general. In true 'carrier' animals as distinct from 'temporary shedders' (Babudieri, 1958) the host animal harbours viable virulent leptospirae in renal tubules of an essentially normal kidney. The leptospirae are excreted in the urine, although the host is itself immune to reinfection with the same serotype of leptospira. There is an unclear relationship between the nature and outcome of the infection, the leptospiral serotype and the host animal. Some species of animals infected with a particular serotype always develop an acute, often fatal, febrile illness, characterized by haemorrhages, renal failure and sometimes jaundice. Other animal hosts infected with the same dose of similar organisms may show no signs of illness but become permanent renal carriers. The ability to develop the renal carrier state is considered to represent a high degree of adaptation of host and parasite to one another. In their natural habitat certain species of host animals in a given locality are always found to carry the same serotype of leptospira (Emanuel, 1959). This concept of 'host of election' is discussed by Uhlenhuth (1943), Alston & Broom (1958) and Babudieri (1958).

A quantitative study of the development of the carrier state in mice has been undertaken. This paper is concerned with some factors influencing the course of infection and determining its outcome.

MATERIALS AND METHODS

Animals

Female Swiss White mice of the University of Sydney stock, allowed unrestricted amounts of commercial mouse pellets and water, were housed at an ambient temperature varying seasonally from 60°–95° F. The mice were never found naturally to carry leptospirae in checks on urine, and on serum for antibody. They were weighed to the nearest 0·1 g. and reached an adult weight of 20–22 g. Urine was collected in a glass capillary from a drop formed at the meatus following abdominal pressure and was examined under dark-ground illumination with a × 20 objective and × 15 eyepiece. Urine was alkalinized when required by injecting intraperitoneally 1–2 ml. 0·15 M $NaHCO_3$ or by substituting this solution for drinking water.

Mice were infected either with cultured leptospirae in their culture medium or with suspensions of mouse kidneys removed rapidly from carrier mice killed by cervical dislocation.

Tissue suspensions

It was found in preliminary experiments that leptospirae were destroyed by sonic vibration at 20 kc./sec. in an MSE-Mullard ultrasonic disintegrator for 30–60 sec. or by blending kidneys with leptospirae at 14,000 r.p.m. in an MSE homogenizer, using 3 ml. cups. Suspensions were therefore prepared by grinding kidneys or other tissues to a paste in a Griffiths tube and making up to a measured volume, usually 8·0 ml., with saline buffered to pH 7·2 with 0·01 M phosphate buffer. Suspensions made in volumes smaller than 8·0 ml. were too dense optically to count leptospirae. The suspensions were left at room temperature for 30 min. or centrifuged at 1000 *g* for 2 min. to settle differentially large tissue particles from leptospirae in the supernatant. Large clumps of leptospirae were not found in the sediment of such preparations. Leptospirae in cultures added to normal mouse kidneys which were then ground as described could be recovered quantitatively. The supernatant was pipetted off, the leptospirae counted and suitable dilutions made in buffered saline.

Leptospirae

The strains of *L. australis* B, and *L. grippotyphosa* were kindly provided by Mr D. J. W. Smith of Brisbane. The other strains were those used in previous studies (Faine, 1957*a*) where the techniques of culture, counting and agglutination-lysis were described.

RESULTS

Patterns of infection in young mice

Groups of 6 mice weighing an average of $14{\cdot}0 \pm 0{\cdot}2$ g. were infected intraperitoneally with 1·0 ml. of culture containing approximately 10^8 leptospirae of one of 4 serotypes, *L. australis* B, *L. grippotyphosa*, *L. australis* A or *L. icterohaemorrhagiae*. The growth curves of the infected mice fell into 2 distinct typical patterns (Fig. 1). Fig. 1 (B), referred to subsequently as 'pattern B', represents the rapid loss of weight found in acute fatal leptospirosis. The fate of the animals is determined within 2 days of injection. Fig. 1 (C), referred to as pattern C, shows the lag in growth during the establishment of a carrier state. The patterns, confirmed in numerous similar experiments, were characteristic irrespective of serotype.

Doses and virulence

The response to various doses of *L. australis* B was assessed by infecting mice intraperitoneally with leptospirae in diluted suspensions of kidneys from carrier mice. Over a period of 2 years it was found that for mice of a given weight the virulence of the leptospirae from kidneys of numerous carriers did not vary. In a typical experiment infecting mice of $12{\cdot}8 \pm 0{\cdot}4$ g. approximate values were MID = 10^2, ID 50 = 5×10^2, LD 50 = 5×10^4, while the dose which infected all mice but killed none (ID 100 = LD 0) was 10^4 leptospirae. The growth curve of mice infected with more than 1 ID but less than 1 LD was pattern C, while that of mice injected with many ID, equivalent to 1 or more LD, was pattern B.

Occasionally in titration experiments mice injected with the largest numbers of leptospirae appeared to be relatively protected. Groups of 6 mice of average weight 12·2 ± 0·4 g. were infected with serial tenfold dilutions of a kidney suspension containing 2×10^5 leptospirae/ml. Each mouse received 1·0 ml. of the appropriate dilution. After 4 weeks 1 mouse had died in each of the groups injected with 2×10^5 and 2×10^4 leptospirae, 3 of the survivors were serologically positive and none were carriers. In the group injected with 2×10^3 leptospirae 2 died, and all 4 of the survivors were serologically positive, 3 of them also carriers. In the 2×10^2 group there were no deaths, 4 were serologically positive and of these 1 was a carrier. Lower doses failed to infect. This observation is attributed to the transfer of protective amounts of antibody with the larger numbers of leptospirae in the 1·0 ml. volumes of the lower dilutions of the kidney suspension, in which the antibody titre was not measured. Titres of undiluted kidney supernatants measured in other experiments were usually 1/2–1/8, once 1/16. A similar complication of the use of urine containing antibodies for the diagnostic inoculation of guinea-pigs was reported by Stuart (1956).

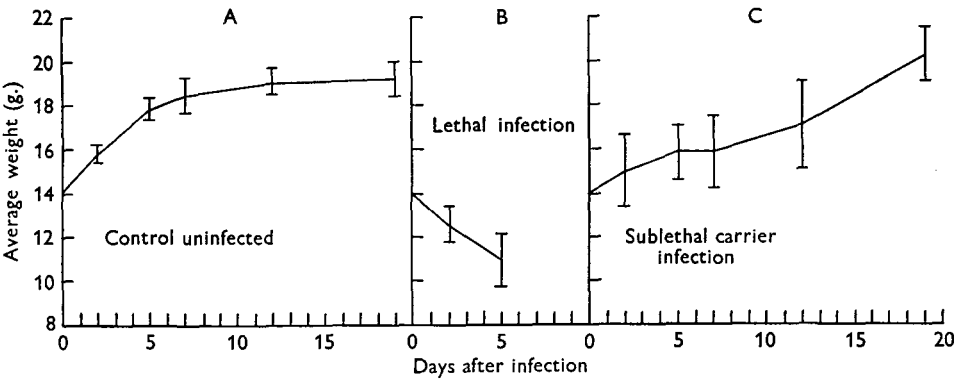

Fig. 1. Growth curves showing average weight ± standard error from a typical experiment where three groups of 8 mice were infected intraperitoneally with 10^8 cultured leptospirae. A, Control uninfected mice. B, Infection with *L. australis* B of high virulence, representing approximately 2 LD 50 in the dose given. C, Infection with *L. icterohaemorrhagiae*. Similar patterns were seen on infection with less than 1 LD 50 of *L. australis* B. These mice became carriers.

After approximately 30 passages in culture *L. australis* B of the same strain as those maintained in mouse kidneys lost virulence. The ID 50 and LD 50 were $> 10^8$ for mice of approximately 12 g. Growth pattern B was never observed, but mice which became carriers after injection with > 1 ID of leptospirae showed a pattern C growth curve.

Influence of age of animal

Infection with L. icterohaemorrhagiae (*Field*)

Adult or weanling mice weighing 12–14 g. were injected intraperitoneally with 10^8 virulent *L. icterohaemorrhagiae* (Field) in their third culture passage. Retardation of weight gain and some carriers were produced. Ten newborn mice weighing 2·2–3·0 g. were injected intraperitoneally with 0·1 ml. containing 10^7 leptospirae

of similar virulence. These mice died in 4–6 days with typical jaundice and haemorrhages of acute leptospirosis. One of 4 mice weighing approximately 9·0 g. died of acute leptospirosis when injected with 10^8 *L. icterohaemorrhagiae* of similar virulence.

Infection with L. australis *B*

In experiments to follow the course of infection with *L. australis* B reported in the following paper (Faine, 1962) 57 mice weighing $12{\cdot}6 \pm 0{\cdot}2$ g. were infected intraperitoneally with 8×10^4 *L. australis* B in 0·1 ml. of a 1/500 dilution of kidney suspension from infected mice. Thirteen mice survived and became carriers, 26 died of acute infection and 20 were killed during the course of the experiment. At the time of infection the 13 which subsequently survived weighed $13{\cdot}0 \pm 0{\cdot}4$ g. and the 26 which died weighed $11{\cdot}8 \pm 0{\cdot}4$ g., a difference of $1{\cdot}2 \pm 0{\cdot}5$ g. ($P = 0{\cdot}02$).

The results of these and other comparable experiments where similar results were obtained showed an approximately linear increase in the proportion of survivors with increasing weight at infection, from approximately 20% survivors among mice of 10·5 g. to 60% among mice of 14·5 g. ($P < 0{\cdot}05 > 0{\cdot}02$) for a group of 85 mice weighing from 9–15 g. This relationship extrapolated shows 100% survival at approximately 19·0 g., at which adult weight mice did not in fact die from acute infection with the doses used. These results show that the age or weight of a mouse determine the course of infection, and, in turn, whether or not the mouse becomes a carrier.

DISCUSSION

The temporary convalescent shedder of leptospirae is analogous to the convalescent carrier in other infections, while the permanent excretor is a true healthy carrier not necessarily showing residual lesions from the acute infection (Babudieri, 1958). In any quantitative experiments on the development of the carrier state it is essential to know that all animals including those which may not become carriers did in fact develop an acute infection. Loss of weight is more specific and therefore preferable to febrile response as an indicator of infection. Neghme, Christen, Jarpa & Agosín (1951), and Emanuel (1959) have described changes in weight in adult mice following infection with leptospirae. A much more sensitive indicator of infection is the failure of young mice to gain weight comparably with controls.

The growth curves of mice weighing 9–15 g. infected with various serotypes fell into two patterns of response, clearly differentiating potential carriers from uninfected mice (Fig. 1). Although these growth curves are averages for groups of mice, the growth curves for individual mice followed closely the trend shown by the average. Increasing the age, represented by the weight at the time of infection, made mice relatively resistant to a standard dose of *L. australis* B, changing their growth curve pattern from B to C, and decreasing mortality. Also newborn mice infected with *L. icterohaemorrhagiae* died while older mice became carriers. These findings show that a factor associated with increasing age alters the nature of the response to infection. This factor could be the rate of production of antibody

as suggested for infection of guinea-pigs with *L. icterohaemorrhagiae* (Faine, 1957*b*).

Similar changes in the growth curves from pattern B to C and vice versa could be obtained by altering either the numbers or the virulence of the infecting leptospirae. Virulence expressed as LD 50 represents the effective dose of infecting organisms able to grow in the host and produce lesions (Faine, 1957*b*; Meynell & Meynell, 1958). Virulence was found not to vary among mouse urinary leptospirae, so that the number of effective virulent organisms inoculated is a second factor which may determine whether or not a susceptible animal becomes a carrier host.

In nature, passive immunity of maternal origin may protect young mice. If mouse antibody has a half-life of about 2–4 days (Weigle, 1957; Humphrey & Fahey, 1961) a mouse born with a protective antibody titre of 1/1000 may be protected against lethal infection for up to 20–40 days, for Smith (1937) showed that a lytic antibody titre of 1/6 was protective. Mice infected during this period are more likely to become carriers than to die if they are infected with doses of leptospirae to be expected in natural surroundings. These observations support the reasons suggested by Babudieri (1958) to explain the age-incidence of rodent carriers of natural infections.

It is difficult to know the infecting numbers of leptospirae in natural infections, but 10^6–10^7 motile and presumably viable leptospirae/ml. have been found in urine from carriers (unpublished observation). Under favourable natural conditions an inoculation of about 10^{-3} ml. of urine, containing up to 10^3–10^4 leptospirae, would be a reasonably large dose. This dose is of an order comparable with the ID 100 of 10^4 leptospirae found experimentally for mice of the age under discussion. Smaller doses could give rise to immunity but not necessarily a carrier state, as seen in the group of mice infected with 2×10^2 leptospirae where 4 of 6 mice were seropositive (titres 1/1000–1/1600) while only 1 of those 4 was a carrier (titre 1/800). Similar results have been obtained in other similar experiments where 10^2–10^3 leptospirae have been inoculated. Obviously a seropositive animal in a survey does not necessarily mean a carrier, and the carrier rate might be much lower than the incidence of seropositive animals might indicate.

Two other inter-related variables determining the outcome of leptospiral infection are the susceptibility of the host animal and the serotype of the infecting leptospirae. Broadly the 'host of election' hypothesis is based on the constant association in a carrier state of a particular leptospiral serotype with a certain species of mammalian host in a natural environment (Broom, 1953; Babudieri, 1958). This association is so constantly observed in Europe that it has been held to represent a fundamental biological relationship (Uhlenhuth, 1943; Babudieri, 1958). It has been shown above that in experimental infections and probably in natural surroundings the factors deciding whether a mouse dies or becomes a carrier are a factor associated with age, specific immunity and the number of infecting leptospirae. The ability of the leptospirae to grow *in vivo* cannot be questioned because a phase of systemic infection always precedes the localization of leptospirae in the kidneys of carriers (Proehoeman, 1930; Faine, 1962), and because of the definition and nature of the carrier state. Thus the only factor

concerned in virulence that varies among the serotypes of leptospirae is pathogenic activity damaging the host; for convenience this may be called 'toxicity' without prejudice as to the nature or mechanism of such activity.

The pathology and pathogenesis of the acute illness and of the carrier state are essentially similar in all mammals, including man (for the acute infection), and following infection with all serotypes of leptospirae (Alston & Broom, 1958; Rimpau, 1950). However, a satisfactory explanation of the toxicity causing the essential lesions of acute leptospirosis is not available. It is necessary to postulate that every serotype is able to produce a qualitatively similar or identical pathogenic mechanism which produces similar pathology in any susceptible mammal. There are no known significant metabolic differences related to serotype among the pathogenic leptospirae.

An alternative, unlikely, hypothesis would propose that each serotype of leptospira has its own characteristic pathogenic mechanism producing lesions solely in one animal or group of animals, the 'host of election'. The lesions produced in the acute infection by all serotypes in all hosts are essentially similar (Rimpau, 1950). Thus this hypothesis requires that lesions common to infection with all serotypes are produced by a different mechanism in each combination of serotype with its 'host of election', and that there are as many different ways of producing the same lesions as there are of such combinations.

This unlikely alternative becomes even less attractive when the wide range of occasional overlaps of serotype with carrier host is examined (Alston & Broom, 1958; Gordon Smith, Turner, Harrison & Broom, 1961*a*, *b*, *c*). It is much more likely that quantitative rather than qualitative adaptation of leptospiral activity and host factors govern the development of the carrier state in individual animals and that the emergence of a 'host of election' in the field is the result of ecological and geographical chances of association between host and leptospiral serotype (Gordon Smith *et al.* 1961*a*, *b*, *c*).

The wide ranges of amount of postulated toxicity and of resistance may result in all grades of infection from extreme susceptibility to extreme resistance between or within species, represented by growth curve patterns A, B or C and all shades between them. This may be formulated

$$\text{Result of infection} = \frac{N \times P}{abR^s},$$

where N = number of organisms able to grow *in vivo*, P = pathogenic activity or 'toxicity', R = resistance to infection, affected exponentially by s, the animal species, and arithmetically by a, age of the animal and b, the specific immunity. A similar type of formula was used by Rich (1951) to interpret factors affecting the production of lesions in tuberculosis. Where $NP > R$, death results. Where $NP = R$, the carrier state results, a condition of equilibrium. Where $NP < R$, the animal resists infection. Applying this to the example of infection with a standard N of *L. icterohaemorrhagiae* in newborn mice, where a is very small and $b = 0$, death resulted because the P of *L. icterohaemorrhagiae* is very large. As a becomes larger, R approximates NP and carriers develop. The same relationships

hold for the infections with *L. australis* B reported here. Using the example quoted by Babudieri (1958) the rat, which has a large value for s, becomes a carrier of *L. icterohaemorrhagiae* (large P) but not *L. canicola* (small P). In young guinea-pigs (small a and s), which are not susceptible to *L. canicola*, infection with *L. icterohaemorrhagiae* is fatal, but as a increases, so does their R. However, *L. canicola* may produce fatal infections in hamsters, which have a very small s, and are susceptible to numerous serotypes (Alston & Broom, 1958), and so this argument continues to the conclusion that *Meriones* sp., which have an extremely small s, are susceptible to most serotypes including those with a very small P (van der Hoeden, 1954).

Thus a 'host of election' hypothesis is tenable if it is based on quantitative considerations rather than qualitative. 'Adaptation' of host to leptospirae can only be a result of the NP relative to R, governing the outcome of the acute infection which precedes establishment of the carrier state. The nature of P, R and s are unknown. For a given species of animal of standard resistance R^s, a and b can decide whether death or carrier status will result from infection.

SUMMARY

The growth curves of young mice experimentally infected with *L. australis* A, *L. australis* B, *L. grippotyphosa* or *L. icterohaemorrhagiae* fell into two types, a 'lethal' pattern of continued weight loss until death, or a 'carrier' pattern of retardation of weight gain. The 'carrier' pattern could be changed to the 'lethal' by increasing the size of the infecting dose of a given serotype, or vice versa. Infection with *L. icterohaemorrhagiae* in newborn mice was fatal, while adults became carriers; susceptibility to infection with *L. australis* B decreased with increasing age.

The numbers of infecting leptospirae and the age of the mouse determined the outcome of infection with a given serotype in a standard test animal. The basis of species susceptibility is unknown, but it is suggested that variation in the ability to produce lesions is the basis of the differences in virulence between serotypes. The 'host of election' in leptospirosis is regarded as the result of a quantitative rather than qualitative adaptation of host and parasite to one another.

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