Epidermal tissue as a primary site of replication of lymphocytic choriomeningitis virus in small experimental hosts

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SUMMARY

When fresh urine from LCM tolerantly infected mice was applied to small areas of excoriated skin of guinea-pigs undiluted or diluted 10^{-1}, a high LCM infectivity developed in the local dermal tissue within 3 days and quickly spread to the lymphatic system. The skin at this site of infection became erythematous 10–12 days after infection and a few days later a rash was often seen in the hairless skin around the mammary teats. A viraemia was first detected at about 8 days after infection and persisted for at least 8 days, during which time a high infectivity titre in skin not only at the infection site but also distal to it suggested that there was a generalized active infection of the dermis. Infectivity in the tongue was simultaneously high and probably associated with erosions of the tongue tip seen a day or two later than the teat rash.

In similar experiments in hamsters and rabbits, indications were again that lightly injured dermis was a primary site of virus replication. These observations should lead to the dermal route receiving greater attention as a potential route of infection of man when exposed to infectious excretions of reservoir hosts of arenaviruses.

INTRODUCTION

Small mammals are likely to play an important part in field investigations with arenaviruses causing disease in man. Virus–host reactions of the prototype virus of this virus group – lymphocytic choriomeningitis (LCM) virus – could influence the approach to such studies. We have found that the natural LCM infection in tolerantly infected mice is readily transmitted to mice, hamsters, rabbits and guinea-pigs by the saliva through a bite, or by the urine through contamination of traumatized skin or buccal mucosa. Investigation of the preliminary stages of virus replication in such infections revealed that the local dermal tissue can be a primary site for replication and the evidence for this is now presented.

METHODS

Experimental hosts

Syrian hamsters, Dutch rabbits and the Pirbright strain of albino guinea-pigs were used.

Weaned hamsters were caged singly or in groups of up to 4 litter-mates if they were compatible and in the same experimental group.

Rabbits were caged singly except for the group infected when 19 days old. These were litter-mates and remained together until weaned at the age of 6 weeks.

The guinea-pigs in Table 5 were housed three to a bin. In the last seven experimental groups in this Table, most of the animals weighed > 900 g. In nearly all the other groups there were equal numbers of animals within the weight ranges 250–350 and 450–550 g. These two ages responded similarly and the results are pooled. Rectal temperatures of 59 of those animals > 450 g were recorded each morning. A recording > 39·2 °C was considered a significant response (Skinner, 1957). Full precautions were taken to minimize cross-contamination between individual guinea-pigs or experimental groups and invalidation of the results through cross-infection is thought to be unlikely in view of the time of onset of clinical signs and the responses detected in controls to infection from the environment and to close contact with infected guinea-pigs. The guinea-pigs in Table 6 were housed singly.

The routine procedure in the guinea-pig experiments was to confirm the specificity of fatal infections by testing for a viraemia. Non-fatal infections were identified by killing the animals 4-5 weeks after infection and testing in mice for a viraemia and for serum neutralizing antibody. Controls kept in the same room were simultaneously submitted to the same tests. In the group exposed to P(PTI) mice in Table 5, four mice shared a bin with two guinea-pigs and the latter were tested 9 days after removal of the mice. In the groups exposed to infected guinea-pigs, individuals shared a bin with two donors from the day of their inoculation. Donors were removed on the 14th day when they were showing severe clinical signs. The contacts were tested 3 weeks after removal of these donors.

Virus source

In all experiments the virus originated from a pool of freshly killed mice of the Pirbright P(PTI) mouse strain in which a tolerant LCM infection originating naturally from a wild source has been maintained for at least 10 years by congenital transmission (Hoyland, Knight & Skinner, 1972; Skinner, Knight & Grove, 1977). The infectivity of P(PTI) mouse serum, saliva and urine was about 10^4 mouse ID50/0·05 ml. All three of these virus sources were used to infect guinea-pigs in the earlier unpublished experiments summarized in Table 5. In all other tabulated experiments mouse urine, the most ubiquitous natural source, was used.

Inoculation techniques

(a) *Scarification.* In hamsters and rabbits the buccal surface of the lower lip opposite the incisors was scratched with a 27 G inoculation needle attached to a syringe containing the inoculum, 0·05 ml of which was then dropped on to the injured area. The skin over the sternum of hamsters was lightly scarified and 0·05 ml of inoculum smeared over it with a smooth glass surface.

(b) *Excoriated skin.* In rabbits and guinea-pigs an area about 20 mm² on the medial surface of the right forearm at the level of the elbow was depilated by repeated application of fresh 20 mm wide Scotch tape (Sellotape Products Ltd., Edgware, Middlesex). Application was continued until a slight exudation of

lymph was evident from failure of the tape to adhere; 0·05 ml of the inoculum was then smeared over the site. The same site on the left forearm was left untouched as a control. Additional sites tested as controls were the hairy skin on the flanks adjacent to the mammary glands and over the lumbar region. In guinea-pigs the local prescapular and axillary lymph nodes were pooled to represent those draining the infection site.

Preparation of tissues

Separate sterile instruments were used for each tissue collected and external tissues – lip, tongue and skin – were washed in buffer before being minced with fresh scissors. Lymph nodes and hamster spleens were ground in a mortar with sand, and other tissues in a sealed Silverson homogenizer (Silverson Machines Ltd., Chesham, Bucks), to give 1/10 to 1/20 suspensions in 0·04 M phosphate buffer, pH 7·6. After centrifugation the supernatants were titrated in mice without delay.

Virus titration

For infectivity titrations 10-fold dilutions in buffer were inoculated by the foot-pad method into mice of the Pirbright P(SD) strain. Local reactions on individual foot pads were counted for the estimation of 50 % end-points by the method of Reed & Muench. Infectivity titres are expressed as $\log_{10}$ ID50/0·05 ml or 0·05 g. Test mice failing to react to the lowest dilutions were challenged 3 weeks after infection and if more than one mouse was found to be immune the tested tissue was assumed to have had a trace (Tr) of infectivity. No infectivity at a 10^{-1} dilution is expressed in the Tables as Neg.

RESULTS

Mice

Failure to transmit LCM infection from P(PTI) mouse saliva or urine through contamination of intact mouse skin or buccal mucosa has been previously reported by us. Infection was, however, readily transmitted by P(PTI) mouse bites or by pin pricks made through skin contaminated with P(PTI) mouse urine. In addition, local viral replication was demonstrated at sites on the tail which were contaminated with P(PTI) mouse urine after light scarification (Skinner & Knight, 1973).

Hamsters

The results in 5-week-old and adult hamsters are presented in Tables 1 and 2. Within 24 h of the contamination of the scarified skin or lip there were indications of the multiplication of the virus in local tissue with titres building up during the first 5 days after infection. A marked drop in titre was evident by the 14th day and virtual extinction of infectivity by the 21st day.

Lymph nodes at three separate sites were tested and, while the local node showed the highest titres at first, high infectivity in the other two showed that there was later a generalized infection of the lymphatic system which persisted to 3–5 weeks after infection.

Table 1. *Hamsters – 5 weeks old – infected by the application of PTI mouse urine into scarified skin overlying the sternum. Local development and persistence of infectivity and its spread to other sites*

Infectivity ($\log_{10}$ ID 50/0·05 g) of tissues from single hamsters killed 1–35 days after infection

Tissues, etc.	1 d	2 d	3 d	3 d	5 d	5 d	7 d	14 d	21 d	35 d	35 d
Scarified sternal skin	3·7	4·6	≥5·8	4·3	4·8	5·3	3·3	2·0	Neg	Neg	Neg
Adjacent control skin	Neg	Neg	Neg	Neg	1·8	3·0	3·0	1·8	Neg	Neg	—
Lymph nodes											
Axillary	Neg	3·5	≥5·5	4·3	4·3	≥5·5	4·5	≥5·3	1·0	Tr	Neg
Submaxillary	Neg	≥3·5	Tr	1·0	4·3	≥5·5	3·8	4·8	2·8	Tr	Neg
Mesenteric	Neg	Neg	Neg	Neg	4·3	4·5	2·3	1·7	1·0	Neg	Neg
Serum	Neg	Tr	Neg	Neg	1·5	2·0	Tr	Tr	Neg	Neg	Neg
Brain	—	—	Neg	Neg	Neg	1·0	Neg	Tr	Neg	—	—
Kidneys	Neg	Neg	Neg	Neg	1·5	2·8	2·3	2·7	Tr	Neg	Neg
Urine	—	Neg	Neg	Neg	—	Tr	Neg	Neg	—	Neg	Neg
Salivary glands	Neg	Neg	Neg	1·4	2·2	2·2	1·0	1·0	Neg	Neg	Neg
Lower lip	—	Neg	Neg	Neg	1·7	1·7	Tr	1·3	Neg	—	—

Table 2. *Hamsters – 3–7 months old – infected by the application of PTI mouse urine to the scarified lower lip: local development and persistence of infectivity and its spread to other sites*

Infectivity ($\log_{10}$ ID 50/0·05 g) of tissues from single hamsters killed 1–35 days after infection

Tissues, etc.	1 d	2 d	3 d	5 d	7 d	10 d	14 d	21 d	28 d	28 d	35 d
Lower lip	2·4	5·2	≥4·9	≥4·9	3·7	4·9	1·9	Tr	Neg	Neg	Neg
Lymph nodes											
Submaxillary	1·5	≥5·5	≥2·5	≥3·5	≥3·5	4·8	4·3	2·5	3·0	Tr	Tr
Axillary	Neg	Tr	≥2·5	≥3·5	≥3·5	4·5	3·5	2·0	Neg	2·2	1·0
Mesenteric	Neg	Tr	1·5	≥3·5	3·3	3·5	1·8	1·0	Neg	Neg	Tr
Serum	Neg	Neg	1·0	1·5	1·0	3·3	Tr	Neg	Neg	Neg	Neg
Spleen	Neg	Tr	≥2·5	≥3·5	2·5	≥3·5	1·0	Tr	Neg	Tr	Neg
Kidneys	Neg	Neg	1·0	2·5	3·0	≥3·5	2·5	3·5	Tr	1·5	1·5
Urine	Neg	Neg	—	Neg	Neg	1·0	Neg	Tr	Tr	Neg	Neg
Salivary glands	Neg	Neg	1·5	2·3	1·3	3·7	1·4	Neg	Neg	Neg	Neg
Mouth swab	Neg	Neg	Tr	Neg	Neg	Neg	Neg	—	Neg	Neg	Neg

Table 3. *Rabbits – 19 days old – infected by the application of PTI mouse urine to the scarified lower lip: local development and persistence of infectivity and its spread to other sites*

Tissues, etc.	Infectivity ($\log_{10}$ ID 50/0·05 g) of tissues from single rabbits killed 3–35 days after infection						
	3 d	5 d	8 d	14 d	21 d	28 d	35 d
Lower lip	3·5	4·2	3·4	Tr	Neg	Neg	Neg
Submaxillary lymph nodes	4·0	3·0	3·5	1·0	Tr	Neg	Neg
Serum	Neg	Tr	Tr	Neg	Neg	Neg	Neg
Spleen	1·4	Tr	Tr	Neg	Tr	Neg	Neg
Kidneys	1·5	2·5	2·3	Neg	Tr	Tr	Neg
Urine	Neg	Neg	Tr	—	Neg	Neg	Neg

Table 4. *Rabbits – 2 months old – infected by the application of PTI mouse urine simultaneously to the scarified lower lip and to excoriated skin inside the right forearm: local development and persistence of infectivity and its spread to other sites*

Tissues, etc.	Infectivity ($\log_{10}$ ID 50/0·05 g) of tissues from single rabbits killed 6–16 days after infection			
	6 d	8 d	12 d	16 d
Lower lip	3·9	2·4	2·4	Tr
Submaxillary lymph nodes	3·0	3·5	3·8	1·3
Stripped skin, right fore-arm	2·7	2·9	3·7	Tr
Right axillary lymph node	2·5	2·0	4·0	Tr
Control skin, left fore-arm	Tr	Neg	1·4	Neg
Left axillary lymph node	—	Neg	3·5	—
Serum	Tr	Neg	Tr	Neg
Spleen	Neg	Neg	Tr	Neg
Kidneys	1·5	Tr	3·0	Tr
Urine	Neg	Neg	Tr	Neg

Infectivity titres in the serum, spleen, salivary glands and kidneys were generally lower than in the lymph nodes. Only traces of infectivity were detected in the brain, urine or mouth swabs. All the infections occurred without marked signs of ill health.

Local multiplication in the skin was confirmed in three other hamsters infected at the age of 2 weeks through scarified sternal skin. The respective titres 1, 2 and 3 days after infection for the local skin were $\geqslant 3·2$, $\geqslant 5·5$, $\geqslant 5·5$, and for serum $< 0·5$, 1·5, 3·5.

Rabbits

The few observations in rabbits – Tables 3 and 4 – showed a general pattern of infectivity in the tissues tested which was very similar to that in the hamster. After evidence of virus multiplication at the infection sites from as early as 3 days after infection and persistence to 12 days, the infectivity waned. Confirmatory evidence was given by the local lymph nodes and at 12 days after infection a control

Table 5. *Summary of earlier experiments in guinea-pigs with LCM virus from PTI mice*

Infectivity of inoculum ($\log_{10}$ mouse ID 50)	Method of exposure to infection	Infection rate	No. of fatal infections*	
Nil	Unexposed controls to following 6 groups	0/28	—	
	Oral	0/6	—	
	Intact skin	0/6	—	
4·8	Scratched skin	6/6	0	
	Intradermal (plantar pad)	6/6	0	
	Subcutaneous	6/6	0	
< 2·0	Mouse bite on lip or abdomen	8/12	0	
—	Close contact for 25 days with adult P(PTI) mice	5/6	1	(11 d)
—	Close contact with two LCM-infected guinea-pigs in next group for 14 days p.i.	1/6	0	
3·5	Intraperitoneal	24/24	24†	
− 0·5	Intraperitoneal	0/5		
0·5	Intraperitoneal	4/6	1	(20 d)
1·5–2·5	Intraperitoneal	14/16	6	(13–20 d)
3·5–6·5	Intraperitoneal	18/18	5	(18–24 d)
1·5–3·5	Intramuscular	11/13	6	(15–19 d)

* Days from exposure to death in parentheses.
† All killed 14 days P.I. when severely infected.

lymph node indicated a generalized infection. Only once did the serum, spleen or urine show more than a trace of infectivity. A higher infectivity was detected in the kidneys. There was no evidence of ill-health.

Guinea-pigs

In earlier work with the Pirbright natural strain of LCM virus, mortality in guinea-pigs was low following infection by many of the routes tested (Table 5). A thermal response always preceded the rapid loss of body condition before death and in many of the non-fatal infections these two clinical signs were also seen. Additional signs which were never seen in uninfected or control animals were erosions of the epithelium round the tip of the tongue, soreness and severe scab formation on the lips, and profuse salivation. The first two of these signs were at predilection sites for generalized lesions following infection with the virus of foot-and-mouth disease and, if specific, suggested an epitheliotropism. Infections were shown to occur readily through scratched skin and P(PTI) mouse bites but not through intact skin or orally (Table 5).

The method of infection used in the present studies was the application of 10^0 to $10^{-2.5}$ P(PTI) mouse urine to skin inside the right forearm immediately after it had been excoriated with Scotch tape. The 50 % infective dilution was about $10^{-1.5}$ ($10^{2.5}$ mouse ID50). The relevant data in the oldest guinea-pigs are in Table 6 and they support the hypothesis that dermal tissue can be a primary site

Table 6. *Guinea-pigs – 700 to > 1000 g – infected by the application of PTI mouse urine to excoriated skin inside the right forearm: local development and persistence of infectivity and its spread to other sites*

Interval P.I.	1 d		2 d		3 d		6 d		7 d		8 d	
Skin at infection site	Neg	—	4·0	2·0	⩾4·5	4·5	⩾4·5	2·5	⩾4·5	4·0	4·5	⩾5·5
Local lymph nodes	Neg	Neg	3·0	Neg	⩾4·5	3·8	⩾4·5	5·0	⩾4·5	⩾5·3	⩾4·5	⩾5·5
Skin at control site	Neg	Neg	—	Neg	1·0	Neg	—	Neg	—	0·5	—	1·5
Control lymph nodes	Neg	Neg	Neg	Neg	Tr	Neg	⩾2·5	5·5	⩾2·2	⩾5·5	⩾2·5	⩾5·5
Serum	Neg	Neg	Neg	Neg	3·0	Neg	Neg	Neg	Tr	Neg	1·0	2·3
Spleen	Neg	Neg	Neg	Neg	—	Neg	⩾4·5	—	⩾4·2	—	⩾4·5	—
Flank skin	—	—	—	Neg	—	Neg	—	Neg	—	Neg	—	3·2
Lumbar skin	—	—	—	Neg	—	Neg	—	Neg	—	Neg	—	2·5
Tongue	—	—	—	Neg	—	Neg	—	Neg	—	Neg	—	4·0
Limb muscle	—	—	—	—	—	—	—	Neg	—	Neg	—	1·0

Interval P.I.	10 d		12 d	15 d	16 d	21 d*	28 d*	35 d*
Skin at infection site	⩾5·5	3·5	⩾5·5	4·3	5·0	Neg	Neg	Neg
Local lymph nodes	5·0	⩾5·5	⩾2·5	4·5	⩾2·5	Neg	Neg	Neg
Skin at control site	4·8	1·0	⩾5·5	4·3	4·5	Neg	Neg	Neg
Control lymph nodes	⩾5·5	⩾5·5	⩾2·5	4·3	⩾2·5	Neg	Neg	Neg
Serum	3·0	1·0	3·8	1·2	3·5	Neg	Neg	Neg
Spleen	—	—	—	2·5	—	Neg	Neg	Neg
Flank skin	⩾4·5	0·8	—	4·3	4·3	Neg	Neg	—
Lumbar skin	⩾4·5	0·5	—	3·8	⩾4·5	Neg	—	—
Tongue	⩾5·0	3·3	—	3·8	⩾5·0	Neg	Neg	—
Limb muscle	3·0	1·0	—	—	—	Neg	—	—

* Serum tests showed an immune response in the guinea-pigs killed 21, 28 and 35 days after infection.

of replication of the virus. The very high infectivity seen at a later stage in the skin from other sites, as compared with that in the serum, suggests that a generalized dermal infection may also occur and could account for the tongue lesions and rash described later.

In these experimental guinea-pigs (Table 6) a superficial scab of dried serous exudate over the excoriated site on the forearm was shed within 3 or 4 days of infection. For the following 5–7 days the site appeared normal and free from inflammation. During this time, however, there was persistence of a high infectivity in the local skin where it was first detected 2 days after infection simultaneously with enlargement, hyperaemia and infectivity in the local lymph nodes. As early as the third day the control lymph nodes and spleen were on one occasion also highly infective. The skin at the control site and at two other sites was not infective until there was a well-defined viraemia from the 8th day onwards.

Up to 8 days after infection guinea-pigs infected by this method maintained their bodily condition, although a few showed transient rises in rectal temperature to about 39·5 °C. After the 8th day many showed the typical prolonged rise in temperature to above 40 °C and a rapid loss of condition. It was in these guinea-pigs in the main that at about 10–12 days after infection the skin within the limits of the infection area became markedly hyperaemic. Often a day or two later a mild rash was also seen at the borders of the hairless zone around the mammary teats of either sex. After a few days erythema at the infection site receded while tending to become brown in colour and the teat rash disappeared. Erosions on the tip of the tongue occurred a little later than the teat rash and consistently at 13–15 days after infection.

The animals in Table 6 which were killed 12, 15 and 16 days after infection had shown an intense local erythema at 12, 14 and 12 days respectively. Four others with an equally intense reaction died on the 13th–14th days. In many other guinea-pigs the erythema was confined at the infection site to well-defined small areas.

In a further four animals not in Table 6 the excoriated site was also lightly scratched before infection and in these the onset of a local erythema appeared to be advanced by 2 days and was confined initially to the site of the scratch. In three of these animals there was, in addition, a marked local induration.

The skin reactions were also seen in younger guinea-pigs (200 and 450 g) infected simultaneously with those in Table 6. Thirty-three guinea-pigs were observed later than 11 days after infection. Eighteen of them developed a local erythema, 11 of which also had a teat rash and 8 a tongue erosion. In all of these 18 an infection with the virus was confirmed by deaths 13–17 days after infection (8 animals), by infective tissues when killed 12–16 days after infection (3 animals), by a good complement-fixing-antibody response at 21 days (1 animal), or by good neutralizing and complement-fixing antibody responses at 4–5 weeks (6 animals). In the 15 guinea-pigs in which no dermal lesions were seen these same tests showed one only to be infected. This is strong evidence for the specificity of the skin reactions.

Erosions had not been seen on the tongues tested from the animals in Table 6. Tongue infectivity was high 8–16 days after infection and from the titres of limb muscle it did not appear that this was part of a generalized infection of skeletal

muscle. In the animal killed at 16 days, which was one of those with the highest titre, a local tissue reaction was indicated by a marked blanching of the terminal 3 mm of the tongue tip after death.

DISCUSSION

There were early reports on the ease with which adult mice and guinea-pigs could be infected with LCM virus through lightly scarified skin (Findlay, Alcock & Stern, 1936; Shaughnessy & Milzer, 1939). In carefully controlled experiments with an intracerebrally passaged guinea-pig strain of LCM virus it was later claimed that guinea-pigs were infected through apparently normal intact skin following the application of what was probably at least 10^6 mouse ID50. Local skin reactions were not reported (Shaughnessy & Zichis, 1940). Following infection by the intradermal route, severe local skin reactions were seen in man (Blanc *et al.* 1951) and in rabbits and guinea-pigs (Roger, 1962, 1963). None of these workers investigated the possibility that the skin was the primary site for virus replication. Dermal lesions are a feature of infections in man with other arenaviruses (Junin, Machupo, Lassa) but are rare in LCM infections. A rash was noted in an early fatal case (Smadel *et al.* 1942) and in six recent cases (Biggar *et al.* 1975). One of the latter patients, interestingly in view of our findings in the guinea-pig, developed a transient hyperaemia in the thumb which had been bitten 12 days earlier by an infected pet hamster.

Platt has demonstrated the uptake of extracellular matter at the site of epidermal injury in guinea-pigs and he has reviewed the importance of this phenomenon in relation to viral infections (Platt, 1963). It is suggested that in our own studies in guinea-pigs engulfment activity was responsible for initiating infection of the injured dermal cells from which virus spread to grow to high titres in the lymphatic system and eventually in many other tissues including uninjured epidermal tissue. In the latter, at specific sites (teat skin, tongue tip), visible reactions occurred a few days after a reaction at the primary site of infection. Histo-immuno-pathological studies would clinch the nature of these skin reactions but the probability is that they are due to immune responses such as are seen in many other virus infections characterized by skin lesions (Mimms, 1966). Had these reactions been seen before the studies with hamsters and rabbits were completed, a more careful search for their occurrence in these species would have been made. As it was, only a local thickening of the infected skin was noted. The sequence of spread of local infection, however, appeared to be similar to that in the guinea-pig, but death was never a sequel.

The route of entry of virus in most reported human LCM infections remains uncertain (Lehmann-Grube, 1971; Biggar *et al.* 1975). The demonstration here of viral replication in the skin of small animal hosts emphasizes the possibility that the dermal route is one whereby human infections might arise. The primary reservoir host, *Mus musculus*, and secondary hosts such as pet hamsters (Skinner & Knight, 1976) may be excreting urine with a high virus titre, thus freshly contaminating the environment daily. A breakdown in personal hygiene leading to contamination of insignificant skin or mucosal injuries with fresh LCM infective

urine can on present evidence be considered a serious hazard. The same hazard might arise when other human pathogens of the arenaviridae are likewise excreted by their reservoir hosts.

REFERENCES

BIGGAR, R. J., WOODALL, J. P., WALTER, P. D. & HAUGHIE, G. E. (1975). Lymphocytic choriomeningitis outbreak associated with pet hamsters. Fifty-seven cases from New York State. *Journal of the American Medical Association* **232**, 494.

BLANC, G., BRUNEAU, J., DELAGE, B. & POITROT, R. (1951). Étude comparative de virus de chorioméningite lymphocytaire d'origine humaine (W. E. Armstrong) et animale (pneumopathie du cobaye). *Bulletin de l'Académie nationale de Médecine* **135**, 520.

FINDLAY, G. M., ALCOCK, N. S. & STERN, R. O. (1936). The virus aetiology of one form of lymphocytic meningitis. *Lancet* i, 650.

HOYLAND, F., KNIGHT, E. H. & SKINNER, H. H. (1972). Eradication of lymphocytic choriomeningitis (LCM) virus – a human pathogen – from a mouse breeding colony. *Journal of the Institute of Animal Technicians* **23**, 6.

LEHMANN-GRUBE, F. (1971). Lymphocytic choriomeningitis virus. *Virology Monographs*, vol. 10. Berlin: Springer-Verlag.

MIMMS, C. A. (1966). Pathogenesis of rashes in virus diseases. *Bacteriological Reviews* **30**, 739.

PLATT, H. (1963). The susceptibility of the skin and epidermoid mucous membranes to virus infection in man and other animals. *Guy's Hospital Reports* **112**, 479.

ROGER, F. (1962). Étude sur le pouvoir pathogène du virus de la chorioméningite lymphocytaire. I. Réactivité dermique chez le lapin. *Annales de l'Institut Pasteur* **103**, 639.

ROGER, F. (1963). Études sur le pouvoir pathogène expérimental du virus de la chorioméningite lymphocytaire. II. Inoculation dans le derme du cobaye. *Annales de l'Institut Pasteur* **104**, 274.

SHAUGHNESSY, H. J. & MILZER, A. (1939). Experimental infection of *Dermacentor andersoni*, Stiles, with the virus of lymphocytic choriomeningitis. *American Journal of Public Health* **29**, 1103.

SHAUGHNESSY, H. J. & ZICHIS, J. (1940). Infection of guinea-pigs by application of virus of lymphocytic choriomengitis to their normal skins. *Journal of Experimental Medicine* **72**, 331.

SKINNER, H. H. (1957). The virus of vesicular stomatitis in small experimental hosts. II. Guinea-pigs and rabbits. *Journal of Comparative Pathology and Therapeutics* **67**, 87.

SKINNER, H. H. & KNIGHT, E. H. (1973). Natural routes for post-natal transmission of murine lymphocytic choriomeningitis. *Laboratory Animals* **7**, 171.

SKINNER, H. H. & KNIGHT, E. H. (1976). The hamster as a secondary reservoir host of lymphocytic choriomeningitis virus. *Journal of Hygiene* **76**, 299.

SKINNER, H. H., KNIGHT, E. H. & GROVE, R. (1977). Murine lymphocytic choriomeningitis: the history of a natural cross-infection from wild to laboratory mice. *Laboratory Animals* **11**, 29.

SMADEL, J. E., GREEN, R. H., PALTAUF, R. M. & GONZALES, T. A. (1942). Lymphocytic choriomeningitis: two human fatalities following an unusual febrile illness. *Proceedings of the Society for Experimental Biology and Medicine* **49**, 683.