PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

How Viruses May Overcome Non-Specific Defences in the Host

A. Kirn and Francoise Keller

Phil. Trans. R. Soc. Lond. B 1983 **303**, 115-122
doi: 10.1098/rstb.1983.0086

Phil Trans. R. Soc. Lond. B **303**, 115–122 (1983)
Printed in Great Britain

How viruses may overcome non-specific defences in the host

By A. Kirn and Françoise Keller

Laboratoire de Virologie de la Faculté de Médecine and Groupe de Recherche INSERM U 74, *Université Louis Pasteur*, 3 *rue Koeberlé*, 67000 *Strasbourg*, *France*

[Plate 1]

The pathogenicity of a virus is mainly related to its capacity to overcome the non-specific defences of a host. Fever limits the multiplication of viruses *in vivo*, thus allowing recovery; thermoresistant strains are virulent because they are capable of escaping the effect of hyperthermia. Resistance to the antiviral effect of interferon does not seem to exist *per se* but interferon may play an indirect role in virus virulence: (i) its effect may be minimized by the destruction of interferon-producing cells such as macrophages; (ii) interferon may render virus-infected target cells resistant to natural killer cell lysis. The interaction of viruses with phagocytic cells plays a predominant role; the ability to grow in macrophages or to induce macrophage blockade or paralysis may constitute a marker of virulence. This is particularly important in the liver in which the infection of Kupffer cells often represents the key event of the disease.

Introduction

Once a virus has infected an organism, the success of the infection is determined by three major events: (i) multiplication of the infecting particles within the primarily infected cells; (ii) spread of the infectious progeny throughout the organism; (iii) replication of the virions in the target cells. In a primary infection, or during the very early stages of a reinfection, inhibition of the multiplication and spread of viruses is mainly produced by non-specific factors. In contrast to the immune defences, which need a certain lapse of time to become operative, the non-specific defences may be effective immediately. The pathogenicity of a virus is therefore in the first place related to its ability to counteract the non-specific defences.

In this paper the main emphasis will be placed on describing how viruses overcome the principal non-specific defences in the host: fever and macrophages, and natural killer cells to a certain extent. Interferon will be mentioned as a mediator of non-specific defence mechanisms, although virulence does not seem to be related to resistance to the antiviral effect of interferon. The section dealing with the interaction of viruses with macrophages will be mainly devoted to the Kupffer cells of the liver, in the light of recent work done in our group.

Resistance to hyperthermia

Protective effect of fever against virus infections

The first report concerning the beneficial effect of hyperthermia on the evolution of a virus disease was from Thompson (1938) who showed that 50% of the rabbits infected with myxomatosis and exposed to ambient temperatures varying between 35 and 38 °C survived,

whereas all the animals subjected to temperatures between 10 and 23 °C died. These observations were confirmed by Marshall (1959), who demonstrated that a small increase in rectal temperature led to a large increase in the number of survivors. Since these first observations, the beneficial effect of elevated temperatures has been demonstrated in different experimental virus infections: poliomyelitis in mice (Lwoff *et al.* 1960), encephalomyelocarditis in mice (Perol-Vauchez *et al.* 1961; Baron & Buckler 1964), herpes in mice (Armstrong 1942; Schmidt & Rasmussen 1960), vaccinia in mice (Kirn *et al.* 1966*a*, *b*), Sindbis in mice (Kirn *et al.* 1967*c*), canine herpes in puppies (Carmichael *et al.* 1969), gastroenteritis in newborn piglets (Furuushi & Shimizu 1976) rabies in mice (Bell & Moore 1974) and the common cold in man (Yerushalmi & Lwoff 1980).

Another approach consisted of studying the effect of different tissue temperatures within the host on the replication of thermosensitive (*ts*) mutants of influenza (Richman & Murphy 1979), parainfluenza (Zygraich *et al.* 1972) and respiratory syncytial virus (Wright *et al.* 1970). It was shown that their multiplication was consistently more restricted within the lungs of mice or hamsters than in their nasal turbinates. The differential replication in the upper and lower respiratory tracts was due to the thermosensitivity of the virus strains, given that a temperature gradient existed within the respiratory tract and that the viruses showed no tissue tropism in organ cultures at 34 °C (Mostow *et al.* 1977). Although there has been only a limited amount of experimental work on the role of fever itself, evidence of its beneficial effect in virus infections was given by Kirn *et al.* (1965*b*). Intratracheal inoculation of the WR strain of vaccinia virus into rabbits provoked a pneumonia accompanied by fever. Treatment of the animals by subcutaneous inoculations of amidopyridine prevented the development of fever in most animals. The proportion of mortality was 2.7 times higher in the amidopyridine treated groups than in the control group. It was also found that the lungs of the apyrexic rabbits contained about 250 times more virus than those of the febrile animals (Kirn *et al.* 1966*a*).

Thermoresistance as a marker of virulence

If body temperature plays a role in the organism's defence by restricting virus multiplication, there should be a correlation between the thermoresistance of a virus strain and its virulence. This was actually found to be so for several viruses, for example, poliovirus (Lwoff *et al.* 1960), foot-and-mouth disease viruses (Prunet 1964) and vaccinia (Kirn *et al.* 1965*a*, 1967*a*; Lab & Kirn 1967). Evaluation of the effects of different strains of influenza virus in humans has revealed that the lower their shut-off temperatures, the more restricted is their replication *in vivo*; viruses with shut-off temperatures of 39 °C were able to produce systemic illness (Murphy *et al.* 1974), whereas the effect of strains with shut-off temperatures of 37 °C was fully attenuated in adults (Murphy *et al.* 1972). Kirn *et al.* (1966*a*, *b*) studied the growth in the mouse brain of three strains of vaccinia virus showing different degrees of thermoresistance. They demonstrated that the higher the thermoresistance, the better the strain multiplied *in vivo* (figure 1). When the mice were exposed to an environmental temperature of 35 °C, which led to an increase in their body temperatures, there was only a small inhibition in the multiplication of the virulent thermoresistant strain whereas the growth of the attenuated thermosensitive strains was completely inhibited. Lwoff & Lwoff (1960) found that the greater the thermoresistance of a strain, the greater its multiplication rate at a given temperature and the smaller the lag phase of its replication cycle. It may therefore be concluded that not only can the thermoresistant strains multiply at elevated body temperatures, but they also demonstrate a

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

better multiplication rate at normal body temperature, which may explain the exacerbation of their virulence. On the other hand, the diminished multiplication rate of the thermosensitive strains renders the infected cells more susceptible to lysis by the immune lymphocytes and may account for their attenuation (Keller *et al.* 1979). However, neither the increased virulence of thermoresistant strains nor the protective effect of hyperthermia could be correlated with variations in interferon synthesis (Lab & Kirn 1967; Kirn *et al.* 1967*b*).

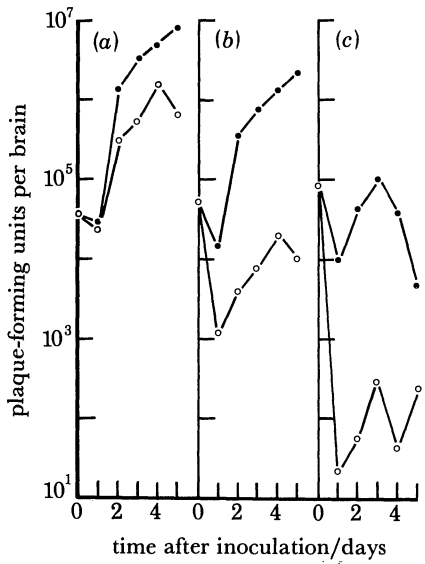


FIGURE 1. Effect of environmental temperatures on multiplication of different strains of vaccinia virus in the brains of mice. (•) Mice kept at 20 °C; (◦) mice kept at 35 °C. (*a*) Thermoresistant strain WR(40); (*b*) thermosensitive strain VP (28); (*c*) thermosensitive strain VP (25). (From Kirn *et al.* (1966), cited in Drillien *et al.* (1978), with permission.)

Restoration of virulence by loss of thermosensitivity

Another argument demonstrating the relation between thermoresistance and virulence is the restoration of virulence that may follow the loss of thermosensitivity (ts^+). For influenza, for example, the loss of the ts phenotype induced either by reversion or by recombination generally results in the recovery of virulence and growth capacity *in vivo* (Richman & Murphy 1979). The replication capacity of clones of three independently derived ts^+ revertants of *ts* influenza virus in the lungs of hamsters was equivalent to that of the wild-type virus which indicates that, for these ts^+ mutants, the *ts* mutation alone is responsible for the restricted replication (Richman *et al.* 1977).

A similar observation was made for vaccinia virus: the occurrence of four cases of vaccinial encephalitis was associated with the isolation in the patients' cerebrospinal fluid of a virus that had lost its ts phenotype and that was able to replicate at 41 °C (Ehrengut *et al.* 1975).

COUNTERACTING MACROPHAGES

Distribution of macrophages within the organism with special reference to Kupffer cells

Fixed macrophages are found in different organs: the liver (Kupffer cells), the lungs (alveolar macrophages), connective tissues (histiocytes), as well as in the spleen, the lymph nodes, the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

bone marrow, and the peritoneal and the pleural cavities. Blood monocytes constitute the free macrophages, which may be recruited in sites of inflammation. Macrophages, which are very efficient phagocytes, also possess a highly developed enzymatic equipment allowing them to digest the material which they have taken up. The endocytosis of foreign particles by macrophages is greatly facilitated by the Fc and C3 receptors they bear at their surface.

The Kupffer cells, given their strategic position in the liver sinusoid, play a major role in the pathogenesis of virus infections. The wall of the hepatic sinusoid is composed of the

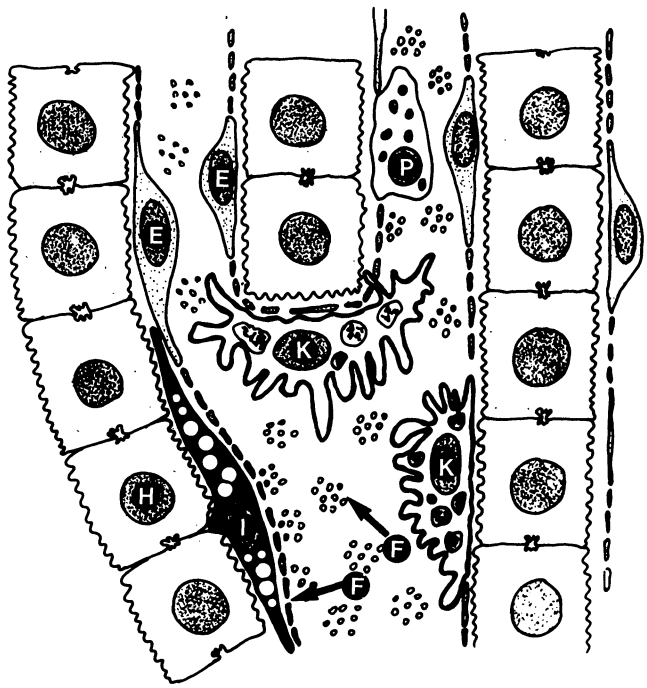


FIGURE 2. Diagram of a liver sinusoid. The wall of the sinusoid is formed by the endothelial cells (E) presenting numerous fenestrae (F) grouped in sieve plates. The fat-storing cells or Ito cells (I) are situated in the space of Disse between the endothelial cells and the hepatocytes (H). The Kupffer cells (K) bulging into the sinusoids either lie on the endothelial lining or are embedded in it. P, pit cell. (From Steffan (1983).)

endothelial cells, which form a continuous layer interrupted only by the small fenestrae in their cytoplasm. Beyond the endothelial cells, in the space of Disse, are situated the fat-storing cells (Ito cells) which store vitamin A in their fat droplets (Wisse & Knook 1979). The Kupffer cells which are scattered along the sinusoids bulge into the lumen; they either lie on the endothelial lining or are embedded in it, sending extensions into the space of Disse (figure 2).

Effect of viruses on the phagocytic and digestive capacities of macrophages

Inhibition of the phagocytic or digestive capacities of macrophages may undoubtedly constitute a marker of viral pathogenicity. However, until now there have been only a few examples of such a type of interference. The infection of primary cultures of rat Kupffer cells with Frog Virus 3, a virus that produces an acute degenerative hepatitis in mice and rats (for a review see Kirn *et al.* (1983)), leads to a rapid inhibition in the phagocytosis of opsonized sheep erythrocytes. The attachment of the red blood cells onto the Fc receptors of the Kupffer cells is not affected but the attached erythrocytes are no longer internalized in virus-infected

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

cells. This inhibition does not extend to pinocytosis because 1 h after infection, at which time the inhibition in phagocytosis is complete, colloidal carbon is still taken up in amounts equivalent to those ingested by control cells (Kirn *et al.* 1980). Kleinerman *et al.* (1975) showed that macrophage chemotaxis was depressed during acute influenza infection. Jakab & Green (1976) studied the ability of alveolar macrophages obtained from Sendai virus-infected mice to take up yeast cells and to prevent phagosome–lysosome fusion. They found that at the beginning of infection there was a delay in the ingestion of the yeast cells accompanied by an inhibition in phagosome–lysosome fusion. Paralysis of phagocytosis, chemotaxis, or digestion of the internalized material not only constitutes a marker of viral pathogenicity but may also favour a secondary bacterial infection.

Replication of pathogenic virus strains in macrophages

Free macrophages infected with viruses may be a source of propagation of infection whereas fixed macrophages may control the invasion of certain organ such as the liver. The multiplication of viruses in macrophages thus constitutes a marker of virulence.

Bang & Warwick (1957) showed that a virulent strain of Newcastle disease virus destroys macrophages in culture whereas the avirulent strain of the same virus grows only poorly in chicken macrophages. Similar results were found with ectromelia (Roberts 1963): an attenuated strain passed only in eggs was less efficient in infecting Kupffer cells than a virulent one passed in mice; no difference though was observed in the ability of the two strains to grow and spread in hepatocytes. Mims (1964) reported that the CL-R strain of vaccinia virus grew in Kupffer cells and thereby gained access to the parenchymal cells whereas the CL strain did not grow in Kupffer cells, which prevented the hepatocytes from becoming infected. It should, however, be stressed that the two previous studies were performed with techniques that did not allow the investigators to recognize the different types of sinusoidal cells and that the conclusions were based on an erroneous representation of the sinusoid architecture (Kirn *et al.* 1982*a*, *b*).

The interaction between mouse hepatitis viruses (MHV) and macrophages has been studied extensively. It has been claimed that the non-pathogenic MHV-1 does not multiply in mouse macrophages whereas the virulent MHV-2 and MHV-3, which provoke a fatal hepatitis in mice, may infect peritoneal macrophages *in vitro* where they multiply to high titres (Bang & Warwick 1960; Mallucci 1965). Using the highly virulent strain of MHV 3, Virelizier & Allison (1976) found a close correlation between the *in vitro* ability of the virus to grow in macrophages from a given strain of mice and its virulence. The same conclusions may apply to herpes viruses (HSV) 1 and 2. Mogensen *et al.* (1974) showed that the intraperitoneal inoculation of HSV2 produced a progressive fatal hepatitis in most strains of mice whereas HSV1 only occasionally provoked a few small foci of liver necrosis. This marker of virulence was found to correspond with the ability of the two viruses to replicate in peritoneal macrophages.

It was found that primary cultures of Kupffer cells, which are the ones that actually control infection of parenchymal liver cells, do not necessarily behave like peritoneal macrophages. So it is that although MHV3 multiplies only poorly in peritoneal macrophages of A/J resistant mice, it grows well in primary cultures of Kupffer cells; the only difference concerning the multiplication in Kupffer cells from both sensitive and resistant mice is the prolonged lag phase found in the former (C. A. Pereira & A. Kirn, unpublished results).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Destruction of the sinusoidal lining of the liver, and its consequences

Some viruses are able to destroy macrophages very rapidly. When this involves Kupffer cells the consequences may be particularly dramatic, as demonstrated by the acute degenerative hepatitis produced by Frog Virus 3 (FV 3) in mice or rats. FV 3 inoculated intravenously is taken up immediately by the Kupffer cells, where it may be found in large amounts. The virus, which is unable to multiply at 37 °C, nevertheless produces a rapid degeneration of the Kupffer and the endothelial cells, which causes breakages in the lining leading to the direct contact of the hepatocytes with the blood (figure 3, plate 1). The sinusoidal cells are thereby prevented from assuming several of their functions relevant to non-specific defence, as follows.

1. *Protective role against secondary viral infection.* Mice or rats with damaged Kupffer and endothelial liver cells become receptive to viruses such as vaccinia or herpes, which are harmless in animals without sinusoidal cell damage (Steffan & Kirn 1979). Furthermore, pretreatment of A/J-resistant mice with sublethal doses of FV 3 renders them sensitive to MHV3 hepatitis (Pereira & Kirn, unpublished results).

2. *Impairment of bacterial endotoxin detoxification.* Bacterial endotoxins are normally present in the portal blood and are continuously detoxified by Kupffer cells (Rutenburg *et al.* 1967). The alteration of these cells by virus infection impedes detoxification, which may thus lead to hepatocellular necrosis (Gut *et al.* 1982).

3. *Depression of interferon synthesis.* Kupffer cells and endothelial cells are capable of producing large amounts of virus-induced interferon (Kirn *et al.* 1982*a*, *b*). The destruction of the r.e.s. in the liver will therefore prevent interferon synthesis, which may favour the spreading of the infection to the parenchymal cells.

These examples clearly show that the ability of a virus to destroy macrophages and especially Kupffer cells undoubtedly constitutes a marker of virulence.

Resistance to NK cell activity

Natural killer (NK) cells, a subpopulation of lymphoid cells, are present in a range of mammalian and avian species. They have a spontaneous cytolytic activity against a variety of tumour cells and some normal cells. Certain cultured cells are relatively resistant to spontaneous cytotoxicity by NK cells but become highly sensitive when virus-infected (Santoli & Koprowski 1979; Welsh & Hallenbeck 1980); the target site recognized by NK cells may be an alteration in the plasma membrane induced by viruses or virus-encoded polypeptides (Rager-Zisman & Bloom 1982).

Interferon seems to be a key immunoregulatory signal for NK activity. Accordingly, in several virus infections of mice, the NK activity has been found to parallel the levels of virus-induced interferon (Welsh 1978). Furthermore, the injection of interferon into mice has resulted in NK activity, whereas treatment with antibody to interferon has prevented the increase in NK cell activity (Gidlund *et al.* 1978). However, the effect of interferon is rather complex because it has been shown that target cells treated *in vitro* with interferon became resistant to lysis by NK cells (Trinchieri & Santoli 1978) and that NK cell activity was depressed *in vivo* by interferon therapy (Ho *et al.* 1981).

The activity of NK cells has been shown to be enhanced in several virus infections of mice such as cytomegalovirus (Bancroft *et al.* 1981), herpes and vaccinia (Piontek *et al.* 1980) and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

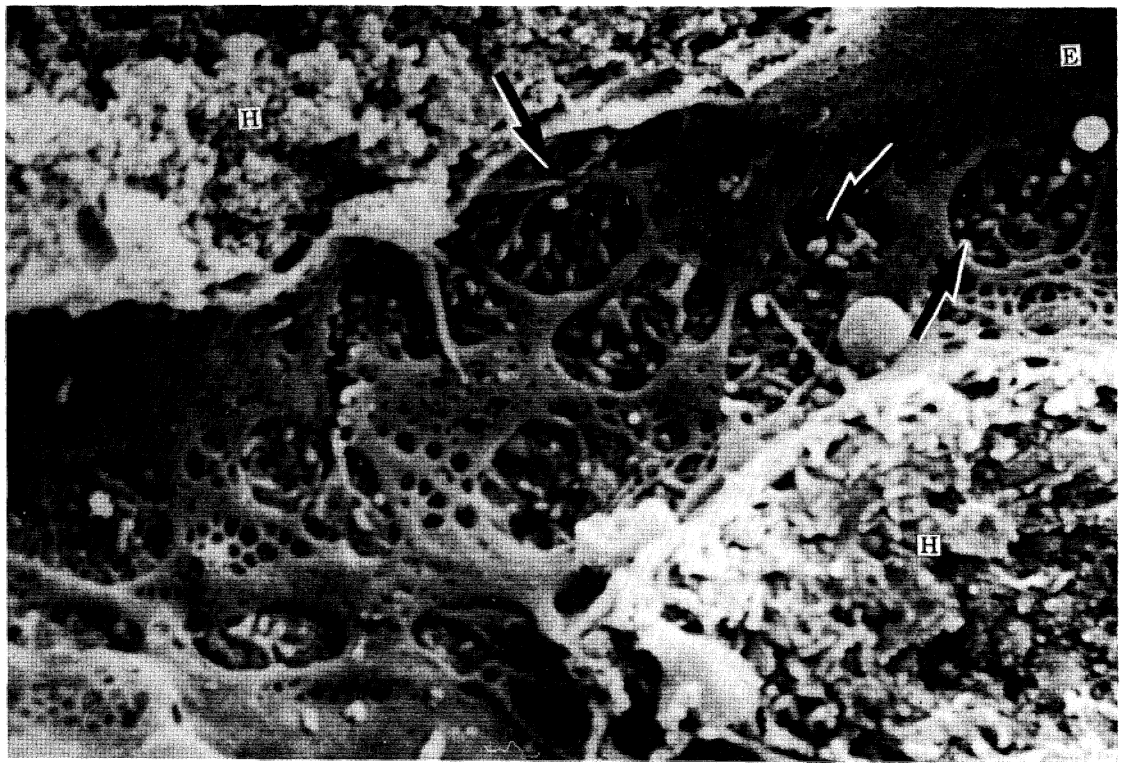


FIGURE 3. Liver sinusoid from a mouse infected for 2 h with a lethal dose of Frog virus 3. Large gaps (arrowed) have appeared in the endothelial cell (E) still recognizable at its fenestrae. The microvilli of the hepatocytes (H) are in direct communication with the sinusoid. Perfusion fixation s.e.m. (Magn. ×17000.)

(*Facing p.* 120)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

lymphocytic choriomeningitis (Welsh *et al.* 1979). Beige mutants, which were shown to have defective NK cells, are susceptible to murine cytomegalovirus whereas their homologous littermates are resistant (Roder & Duwe 1979). Although NK cells do not seem to play a role in experimental virus infections such as mouse hepatitis (Schindler *et al.* 1982) and Sindbis virus infection of mice, it can nevertheless be postulated that the capacity of viruses for counteracting NK cell activity may constitute a factor of virulence.

There are a few examples that support this hypothesis. L 929 cells infected with Sendai, vesicular stomatitis, Sindbis and herpes viruses commonly become resistant to NK lysis (Welsh & Hallenbeck 1980). The binding step of the target cell to the NK cell is not affected. It is possible that interferon produced in these cells mediates this protection. However, Vero cells infected with HSV1 also become resistant to NK cell-mediated lysis (Welsh & Hallenbeck 1980), and their resistance cannot be explained by interferon, because it is not produced by Vero cells. In this example resistance appears to be due to the inability of herpes virus-infected Vero cells to bind to NK cells. Thus virus infection of normal cells may render them resistant to lysis by interferon-activated NK cells.

Conclusion

In addition to fever, macrophages, and NK cells, several other non-specific mechanisms take part in the defence of the organism against virus infection. These include non-specific inhibitors in serum and tissues, low pH or inflammatory exudates, nucleases and proteases in cells. However, it is not known whether strategies are used by viruses to overcome these defences. The fact that some viral strains may be poor interferon-inducers or may produce interferon antagonists (Chany & Brailowsky 1967) could also account for increased virulence. It should be kept in mind that counteracting the non-specific defences of the host does not depend only on the pathogenicity of the virus strain but also on the quality of the host response.

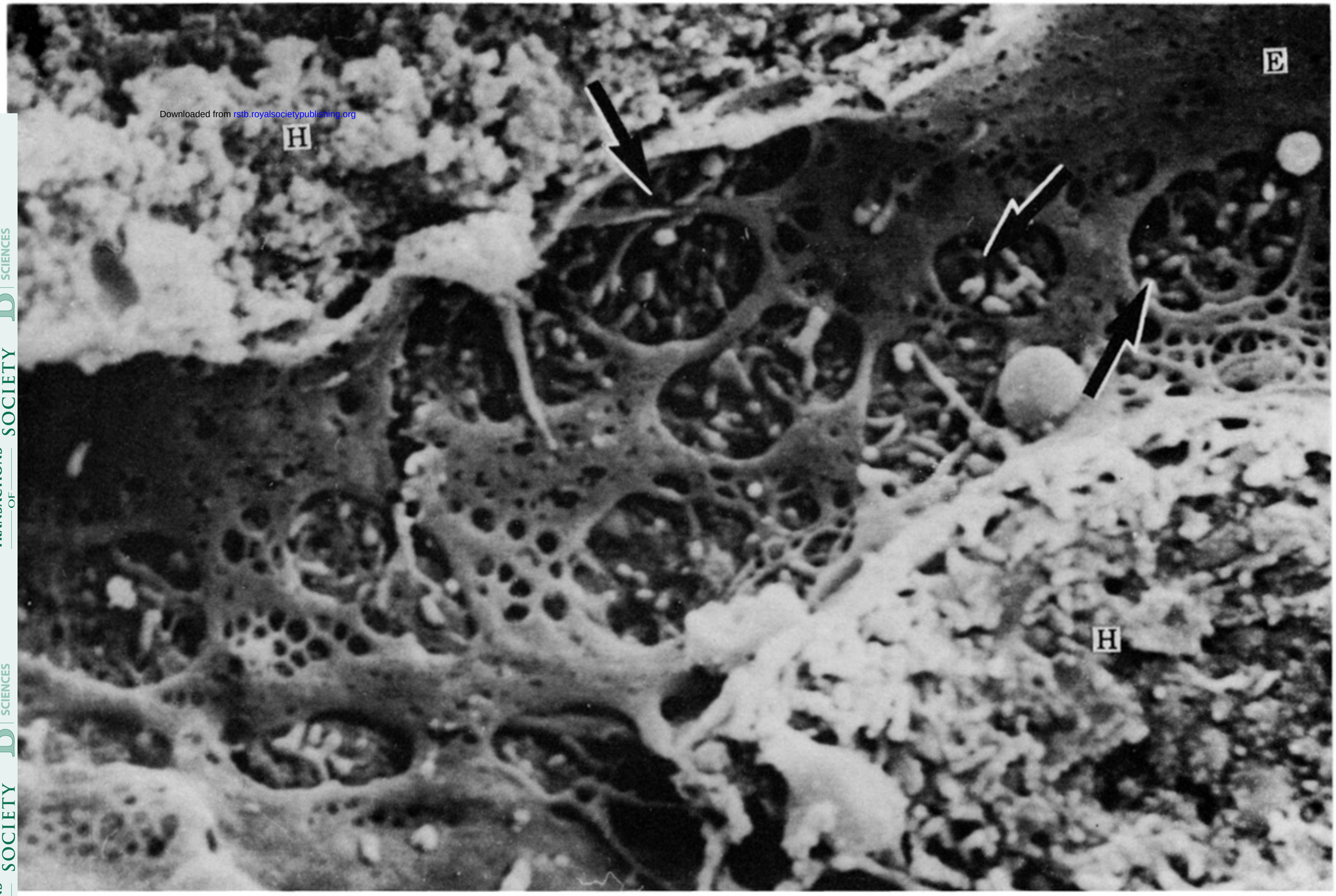
References

Armstrong, C. 1942 *Milit. Surg.* **91**, 129–145.
Bancroft, G. J., Shellam, G. R. & Chalmer, J. E. 1981 *J. Immunol.* **126**, 988–994.
Bang, F. B. & Warwick, A. 1957 *J. Path. Bact.* **73**, 321–330.
Bang, F. B. & Warwick, A. 1960 *Proc. natn. Acad. Sci. U.S.A.* **46**, 1065–1075.
Baron, S. & Buckler, C. E. 1964 *J. Immunol.* **93**, 45–50.
Bell, J. F. & Moore, G. J. 1974 *Infect. Immun.* **10**, 510–515.
Carmichael, L. E., Barnes, F. D. & Percy, D. H. 1969 *J. infect. Dis.* **120**, 669–678.
Chany, C. & Brailowsky, C. 1967 *Proc. natn. Acad. Sci. U.S.A.* **57**, 87–94.
Drillien, R., Keller, F., Koehren, F. & Kirn, A. 1978 In *Viruses & environment* (ed. E. Kurstak & K. Maramorosch), pp. 381–396. New York, San Francisco and London: Academic Press.
Ehrengut, W., Sarateanu, D. E., Alswede, U., Habib, A. & Tetzlaff, G. 1975 *Archs Virol.* **48**, 229–238.
Furuushi, S. & Shimizu, Y. 1976 *Infect. Immun.* **13**, 990–992.
Gidlund, M., Orn, A., Wigzell, H., Senik, A. & Gresser, I. 1978 *Nature, Lond.* **273**, 759–761.
Gut, J. P., Schmitt, S., Bingen, A., Anton, M. & Kirn, A. 1982 *J. infect. Dis.* **146**, 594–605.
Hirsch, R. L. 1981 *Immunology* **43**, 81–89.
Ho, M., White, L. T., Haverkos, H. W., Breinig, M. C., Pazin, G. J. & Armstrong, J. A. 1981 *J. infect. Dis.* **144**, 605.
Jakab, G. J. & Green, G. M. 1976 *J. clin. Invest.* **57**, 1533–1539.
Keller, F., Drillien, R. & Kirn, A. 1979 *Infect. Immun.* **26**, 841–847.
Kirn, A., Braunwald, J. & Dammron, A. 1965*a* *C. r. hebd. Séanc. Acad. Sci., Paris* **260**, 6230–6231.
Kirn, A., Dammron, A., Braunwald, J., Lab, M. & Wurtz, R. 1966*a* *Annls Inst. Pasteur, Paris* **110**, 697–706.
Kirn, A., Dammron, A., Braunwald, J. & Wurtz, R. 1965*b* *C. r. hebd. Séanc. Acad. Sci., Paris*, **261**, 1923–1925.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Kirn, A., Gut, J. P., Bingen, A. & Steffan, A. M. 1983 *Hepatology* **3**, 105–111.
Kirn, A., Gut, J. P. & Gendrault, J. L. 1982*a* In *Progress in liver diseases* (ed. H. Popper & F. Schaffner), vol. 7, pp. 377–392. New York: Grune & Stratton.
Kirn, A., Koehren, F. & Steffan, A. M. 1982*b* *Hepatology* **2**, 676.
Kirn, A., Lab, M. & Dammron, A. 1967*a* *Annls Inst. Pasteur, Paris* **112**, 113–116.
Kirn, A., Schieffer, K. & Braunwald, J. 1966*b* *Annls Inst. Pasteur, Paris* **111**, 645–654.
Kirn, A., Schieffer, K. & Tinland, R. 1967*b* *Nature, Lond.* **215**, 86–87.
Kirn, A., Steffan, A. M. & Bingen, A. 1980 *J. reticuloendoth. Soc.* **28**, 381–389.
Kirn, A., Tinland, R., Lab, M. & Schieffer, K. 1967*c* *Annls Inst. Pasteur, Paris* **113**, 771–780.
Kleinermann, E. S., Snyderman, R. & Daniels, C. A. 1975 *Lancet* ii, 1063–1066.
Lab, M. & Kirn, A. 1967 *Annls Inst. Pasteur, Paris* **113**, 341–350.
Lwoff, A. & Lwoff, M. 1960 *Annls Inst. Pasteur, Paris* **98**, 173–203.
Lwoff, A., Tournier, P., Lwoff, M. & Cathala, F. 1960 *C. r. hebd. Séanc. Acad. Sci., Paris* **250**, 2644–2645.
Mallucci, L. 1965 *Virology* **25**, 30–37.
Marshall, I. D. 1959 *J. Hyg., Camb.* **57**, 484–497.
Mims, C. A. 1964 *Bact. Rev.* **28**, 30–71.
Mogensen, S. C., Teisner, B. & Andersen, H. K. 1974 *J. gen. Virol.* **25**, 151–155.
Mostow, S. R., Flatauer, S., Paler, M. & Murphy, B. R. 1977 *J. infect. Dis.* **136**, 1–6.
Murphy, B. R., Chalhub, E. G., Nusinoff, S. R. & Chanok, R. M. 1972 *J. infect. Dis.* **126**, 170–178.
Murphy, B. R., Hodes, D. S., Nusinoff, S. R., Spring-Stewarts, S., Tiernev, E. L. & Chanock, R. M. 1974 *J. infect. Dis.* **130**, 144–149.
Perol-Vauchez, Y., Tournier, P. & Lwoff, M. 1961 *C. r. hebd. Séanc. Acad. Sci., Paris* **253**, 2164–2166.
Piontek, G. E., Weltzin, R. & Tompkins, W. A. F. 1980 *J. reticuloendoth. Soc.* **27**, 175–188.
Prunet, P. 1964 *Annls Inst. Pasteur, Paris* **106**, 18–28.
Rager-Zisman, B. & Bloom, B. R. 1982 *Springer Semin. Immunopathol.* **4**, 397–414.
Richman, D. D. & Murphy, B. R. 1979 *Rev. infect. Dis.* **1**, 413–433.
Richman, D. D., Murphy, B. R. & Chanock, R. M. 1977 *Virology* **83**, 356–364.
Roberts, J. A. 1963 *Br. J. exp. Path.* **44**, 465–472.
Roder, J. & Duwe, A. 1979 *Nature, Lond.* **278**, 451–453.
Rutenburg, S., Skarnes, R., Palmerio, C. & Fine, J. 1967 *Proc. Soc. exp. Biol. Med.* **125**, 455–459.
Santoli, D. & Koprowski, H. 1979 *Immunol. Rev.* **44**, 125–163.
Schindler, L., Engler, H. & Kirchner, H. 1982 *Infect. Immun.* **35**, 869–873.
Schmidt, J. R. & Rasmussen, A. F. Jr 1960 *J. infect. Dis.* **107**, 356–360.
Steffan, A. M. 1983 Thesis, Université Louis Pasteur, Strasbourg.
Steffan, A. M. & Kirn, A. 1979 *J. reticuloendothel. Soc.* **26**, 531–538.
Thompson, R. L. 1938 *J. infect. Dis.* **62**, 307–312.
Trinchieri, G. & Santoli, D. 1978 *J. exp. Med.* **147**, 1314–1333.
Virelizier, J. L. & Allison, A. C. 1976 *Arch. Virol.* **50**, 279–285.
Welsh, R. M. 1978 *J. exp. Med.* **148**, 163–181.
Welsh, R. M. & Hallenbeck, L. A. 1980 *J. Immunol.* **124**, 2491–2497.
Welsh, R. M., Zinkernagel, R. M. & Hallenbeck, L. A. 1979 *J. Immunol.* **122**, 457–481.
Wisse, E. & Knook, D. L. 1979 In *Progress in liver diseases* (ed. H. Popper & F. Schaffner), vol. 6, pp. 153–171. New York: Grune & Stratton.
Wright, P. F., Woodend, W. G. & Chanock, R. M. 1970 *J. infect. Dis.* **122**, 501–512.
Yerushalmi, A. & Lwoff, A. 1980 *C. r. hebd. Séanc. Acad. Sci., Paris* D **291**, 957–958.
Zygraich, N., Lobmann, M. & Huygelen, C. 1972 *J. Hyg., Camb.* **70**, 229–234.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



GURE 3. Liver sinusoid from a mouse infected for 2 h with a lethal dose of Frog virus 3. Large gaps (arrowed) have appeared in the endothelial cell (E) still recognizable at its fenestrae. The microvilli of the hepatocytes (H) are in direct communication with the sinusoid. Perfusion fixation s.e.m. (Magn. × 17000.)