a mnemonic labeling scheme that relates topologically equivalent portions of different membranes and that can be easily applied in a uniform manner to all freeze-etch images. We urge it be adopted by all who publish freeze-etch results.

DANIEL BRANTON
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138
STANLEY BULLIVANT
Department of Cell Biology, University of Auckland, Private Bag, Auckland, New Zealand
NORTON B. GILULA
Rockefeller University, New York 10021
MORRIS J. KARNOVSKY
Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115
H. MOOR
K. MÜHLETHALER
Laboratory of Electron Microscopy, Swiss Federal Institute of Technology, 2 Universitatstrasse, Zurich, Switzerland
D. H. NORTHCOTE
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, England
LESTER PACKER
Department of Physiology, University of California, Berkeley 94720
BIRGIT SATIR
PETER SATIR
Departments of Physiology and *Anatomy, University of California, Berkeley 94720*
VOLKER SPETH
Max Planck Institute for Immunobiology, Freiburg, Germany
L. A. STAEHLIN
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder 80302
RUSSELL L. STEERE
Plant Protection Institute, U.S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland 20705
RONALD S. WEINSTEIN
Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111

References and Notes

1. R. L. Steere, *J. Biophys. Biochem. Cytol.* **3**, 45 (1957); H. Moor, K. Mühlethaler, H. Waldner, A. Frey-Wyssling, *ibid.* **10**, 1 (1961).
2. D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1048 (1966).
3. L. Benedetti and P. Favard, Eds., *Freeze-Etching Techniques and Applications* (Societé Française de Microscopie Electronique, Paris, 1973).
4. C. de Duve, in *Lysosomes in Biology and Pathology*, J. T. Dingle and H. B. Fell, Eds. (North-Holland, Amsterdam, 1969), vol. 1, pp. 3–40.
5. We thank P. Garwood for help in preparing the figure.

29 April 1975

Enhancement of Mouse Cytomegalovirus Infection During Host-Versus-Graft Reaction

Abstract. *C_3H/He mice chronically infected with murine cytomegalovirus were given skin allografts from histoincompatible BALB/c donors. A significant increase in cytomegalovirus titers occurred within 3 days after placement of the graft in the spleens and kidneys of the allograft recipients as compared with control animals. No significant changes in virus titers were detected in the salivary gland, lung, liver, or blood of allograft recipients. These results indicate that the host-versus-graft reaction alone can enhance murine cytomegalovirus in a chronically infected host and may help explain the high incidence of cytomegalovirus infection seen after renal and other allograft transplantation in man.*

Since the description of cytomegalovirus (CMV) inclusions in the organs of renal transplant recipients at autopsy, numerous studies have documented the occurrence of CMV infections in these patients (*1–4*). Between 73 and 91 percent of renal allograft recipients develop CMV infection after transplantation, as judged by virologic, serologic, and histologic evidence. CMV infections are common in liver allograft recipients (*5*) and in patients given transplants of allogeneic bone marrow (*6*). CMV infection after blood transfusion has been reported frequently, both in patients undergoing extracorporeal circulation and in other transfusion recipients (*7*). While multiple putative risk factors for CMV infection may be operative in these various groups, the feature common to all, the interaction between a host and an allogeneic graft, has been postulated to be the inciting determinant (*8*).

Most CMV infections in transplant recipients appear to be of little consequence (*2, 3*). However, various clinical manifestations including fever, pneumonitis, leukopenia, mononucleosis, and hepatitis have been associated with CMV infection in renal transplant recipients (*3, 9*). Rifkind and his associates (*10*) first described the coincidence of allograft rejection with serologic evidence of CMV infection in two renal transplant patients. Other investigators noted that acute rejection episodes after renal transplantation appeared to follow infections caused by various viruses (*11*). In an extensive study of 61 renal transplant recipients, Lopez and his colleagues (*4*) found that the clinical triad of fever, leukopenia, and allograft rejection was associated with herpesvirus infections, particularly CMV. They concluded that either the virus infection triggered allograft rejection or that the rejection process activated a latent CMV infection. Since it is difficult to differentiate between these two possibilities by epidemiologic studies in man, the effect of a skin allograft on chronic CMV infection in the mouse was investigated.

To establish the course of murine cytomegalovirus (MCMV) infections, groups of mice were examined over a period of from 1 to 23 weeks after receiving virus. Five-week-old C_3H/He mice were inoculated intraperitoneally with 2×10^5 plaque-forming units (PFU) of Smith strain MCMV. At each sampling period, mice were killed by intracardiac exsanguination. The liver, spleen, kidneys, and salivary glands were dissected in toto, and 10 percent individual organ homogenates were made for virus titration. The titers of 10 percent whole, heparinized blood were also determined. Virus titration was performed by the plaquing method with tragacanth overlay (*12*). The virus titers were highest in the salivary gland, followed by that of the spleen and kidneys (Table 1). The salivary glands of all mice contained virus 1 week after infection. Virus titer peaked at 4 weeks and declined thereafter. All spleens were infected 1 week after infection; many were negative at 2 weeks, and no spleens containing virus were detected after 9 weeks. The proportion of positive kidneys was similar. MCMV was found in low titer (log PFU per 100 mg of tissue homogenate was 1.97 ± 0.40, S.E.) in the livers of about one-third of the mice from 1 to 6 weeks after infection. No virus was detectable in the liver from 9 weeks through 23 weeks after infection. Viremia was detected in low titer (< 10 PFU/0.1 ml) in two of six mice infected 1 week previously, but MCMV was not found in the blood thereafter.

The proportion of infected organs decreased at varying rates after the first weeks of infection (also see below). But titers of infected spleens and kidneys varied relatively little during the period from 2 to 6 weeks after infection. This characteristic permitted the use of these organ titers for detection of viral enhancement.

The effect of a skin allograft on chronic infection was first studied in C_3H/He mice inoculated 5 weeks previously with 2×10^5 PFU of MCMV. Normal 10-week-old BALB/c mice served as donors. Full-thickness donor skin grafts of approximately 15 mm in diameter were cut with a

cork borer and sutured into surgically prepared sites of recipients under sodium pentobarbital anesthesia. At intervals, we killed paired experimental and control mice and determined the MCMV titer of individual organ suspensions of each mouse. Control animals received no treatment, sodium pentobarbital anesthesia only, or an autograft in which the skin was merely sutured back onto the same bed from which it was removed. As there was no discernible difference in the organ titers of these three groups, the results of control animals have been combined.

Allografts showed visible signs of rejection after about 7 days and were sloughed completely in about 10 days. Organ titers found in two separate experiments were similar and are presented together in Fig. 1. The proportion of infected spleens of allograft animals (Fig. 1A) was not significantly different from what would be expected from the data on the course of the chronic infection (Table 1), or from the control group. However, the geometric mean virus titers of positive spleens in animals given allografts are consistently higher than the controls from day 1 through day 10 after grafting. The differences in titers are significant by the Wilcoxon rank sum test ($P < .01$).

Similar data on the MCMV titers of the kidneys from the same animals are shown in Fig. 1B. Again the proportion of infected kidneys was not significantly affected by placement of an allograft. But the titers of positive kidneys from allograft animals are increased from day 1 through day 10 after grafting. Comparison of allograft with control mice by the rank sum test is also significant with $P < .01$.

All salivary glands contained MCMV in high titer throughout the course of the experiments. There was no difference in salivary gland titer between allograft and control mice. Virus in low titer was found infrequently in liver and rarely in lung; there were no differences between allograft and control animals with regard to the virus titer in these two organs. Virus was detected in the blood of two animals 3 and 4 days after allograft. No control animals demonstrated viremia.

In addition to mice infected for 5 weeks, separate experiments were done with mice infected for 2 weeks and 13 weeks. Controls in these experiments were mice given an autograft. The virus titers after grafting in the individual spleens of 2-week infected mice are shown in Fig. 1C. The proportion of virus-negative spleens was somewhat higher than expected, so that few positive organs remain for comparison. However, the individual titers of virus-containing spleens from mice receiving allografts are consistently higher than those of autograft animals from day 3 through the remainder of the experiment. The MCMV titers of the individual kidneys from these same mice is shown in Fig. 1D. The higher titers of virus-positive kidneys from allograft animals is again apparent.

Limited data were obtained concerning the effect of skin allograft on infection of 13 weeks' duration because of the paucity of organs that contained virus. None of the spleens from allograft or control animals contained detectable MCMV. The kidney virus titers were again higher 5 and 7 days after allograft placement, as compared to similar titers in control mice. There was also a suggestion, not yet statistically proved, that the proportion of infected kidneys was higher in the animals receiving allografts.

The source and mechanism of enhancement of virus titers in spleens and kidneys of chronically infected mice given an allograft from a histoincompatible donor remains to be explained. The lack of any significant change in the proportion of posi-

Table 1. Organ distribution of MCMV after infection in C_3H/He mice. The fraction (P/T) of organs positive for virus is the ratio of the number of organs containing detectable virus to the total number of animals in each group. The titers are the geometric mean titers of virus-positive organs in log PFU of MCMV per 100 mg of tissue homogenate ± S.E.

After infection (weeks)	Salivary gland		Spleen		Kidney	
	P/T	Titer	P/T	Titer	P/T	Titer
1	6/6	3.7 ± 0.4	6/6	2.4 ± 0.5	4/6	1.2 ± 0.4
2	11/11	6.2 ± 0.3	5/11	3.4 ± 0.6	5/11	1.9 ± 0.7
4	6/6	7.0 ± 0.4	3/6	2.8 ± 0.7	2/6	2.5 ± 0.2
6	6/6	6.2 ± 0.4	1/6	3.0	2/6	2.4 ± 0.5
9	5/6	5.1 ± 0.7	1/6	2.7	0/6	
12	4/6	3.4 ± 0.5	0/6		0/6	
16	3/6	2.9 ± 0.7	0/6		0/6	
23	3/7	3.5 ± 0.5	0/7		1/7	1.9

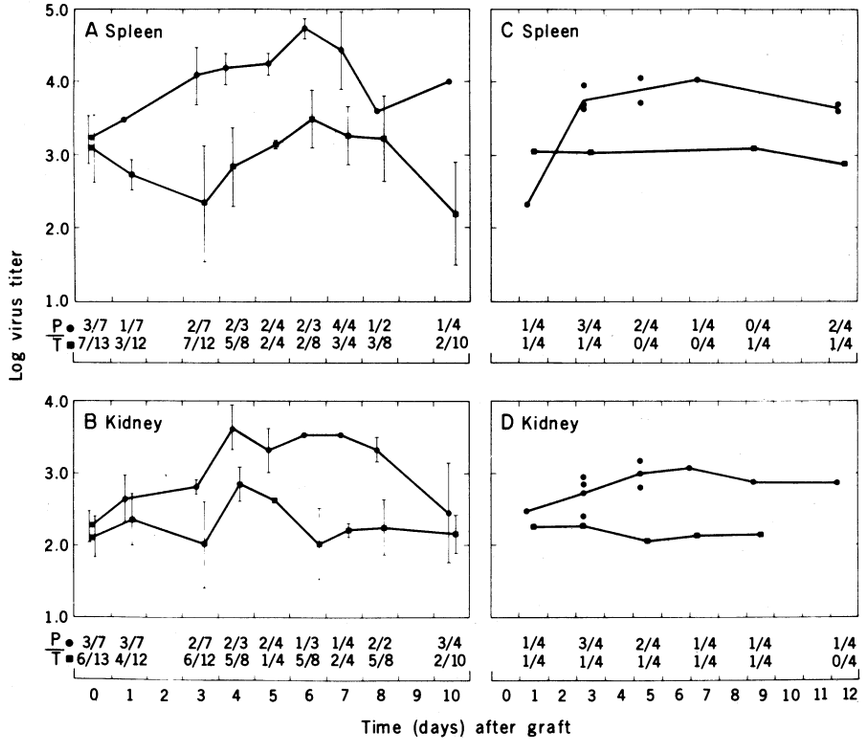

Fig. 1. Effect of an allograft on the MCMV titers in the spleens and kidneys of chronically infected C_3H/He mice. Mice inoculated with 2×10^5 PFU of MCMV either 5 weeks (A, B) or 2 weeks (C, D) previously were given an allograft (●) or control treatment (■). The fraction (P/T) of organs positive for virus is the ratio of the number of organs containing detectable virus to the total number of mice in each group. Each point in (A) and (B) represents the geometric mean titer (± S.E.) of virus-positive organs from allograft and control mice. Each point in (C) and (D) is the organ titer of each individual allograft (●) or control (■) mouse. Titers are expressed as log PFU of MCMV per 100 mg of organ homogenate.

tive organs supports the concept that virus titers are enhanced only in organs that already contain some free virus. Further studies are needed to determine whether any latently infected cells were converted to producer cells. The possibility that skin allograft placement somehow permitted increased seeding of MCMV from other sites such as the salivary glands, where the virus is present in high titer, is considered unlikely. Viremia was rare at this stage of infection and virus titers in other organs, such as the liver and the lung, were not enhanced.

Recently, Olding and his co-workers (*13*) activated and recovered MCMV from the spleen cells of 2- and 5-month-old mice infected in utero or at birth by cocultivation with allogeneic fibroblasts. No virus was detected when disrupted spleen cells were cultured. The latently infected cells were apparently B lymphocytes. These experiments showing activation of MCMV by allogeneic reaction in vitro may represent the counterpart of our findings in the animal.

Mouse leukemia virus has been activated in inapparently infected mice by the transfer of parental spleen cells to an F_1 recipient, producing a graft-versus-host reaction (*14*). Later, Hirsch and his associates (*15*) found that latent leukemia virus was activated by a combination of skin allograft and administration of antilymphocyte serum. The mechanism by which mouse leukemia virus is activated is not known, but there is evidence that activation is not solely a function of lymphocyte blast transformation (*16*).

Lopez and co-workers (*4*) found that CMV infections in renal transplant recipients are temporally associated with graft rejection episodes. They proposed that either the renal allograft activates a latent CMV infection or that the viral infection in some way promotes graft rejection. Evidence indicating that CMV enhances cell-mediated immune responses would support the latter hypothesis. Actually, the opposite appears to be the case in mice. Howard and associates (*17*) found that skin grafts made across strong (H-2) and weak (H-Y) histoincompatibility barriers had prolonged survival times when the recipient mice were acutely infected with MCMV. Graft survival time was significantly increased in mice infected up to 7 days before grafting. In addition, spleen cells harvested from mice infected with MCMV showed decreased uptake of tritiated thymidine in response to phytohemagglutinin and in mixed lymphocyte culture. Our studies show that a skin allograft from a histoincompatible donor enhances MCMV infection in recipient mice. These two observations taken together support the concept that host response to the allograft activates CMV infection in renal transplant recipients, rather than the reverse.

BRIAN C. WU, JOHN N. DOWLING
JOHN A. ARMSTRONG, MONTO HO
Department of Microbiology, Graduate School of Public Health, and *Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261*

References and Notes

1. D. Rifkind, T. E. Starzl, T. L. Marchioro, W. R. Waddell, D. T. Rowlands, Jr., R. B. Hill, Jr., *J. Am. Med. Assoc.* **189**, 808 (1964).
2. J. E. Craighead, J. B. Hanshaw, C. B. Carpenter, *ibid.* **201**, 725 (1967).
3. H. K. Andersen and E. S. Spencer, *Acta Med. Scand.* **186**, 7 (1969).
4. C. Lopez, R. L. Simmons, S. M. Mauer, J. S. Najarian, R. A. Good, *Am. J. Med.* **56**, 280 (1974).
5. V. A. Fulginiti, R. Scribner, C. G. Groth, C. W. Putnam, L. Brettschneider, S. Gilbert, K. A. Porter, T. E. Starzl, *N. Engl. J. Med.* **279**, 619 (1968).
6. P. Neiman, P. B. Wasserman, B. B. Wentworth, G. F. Kao, K. G. Lerner, R. Storb, C. D. Buckner, R. A. Clift, A. Fefer, L. Fass, H. Glucksberg, E. D. Thomas, *Transplantation* **15**, 478 (1973).
7. L. Kääriäinen, J. Paloheimo, E. Klemola, T. Mäkelä, A. Koivuniemi, *Ann. Med. Exp. Biol. Fenn.* **44**, 297 (1966); D. P. Stevens, L. F. Barker, A. S. Ketcham, H. M. Meyer, Jr., *J. Am. Med. Assoc.* **211**, 1341 (1970); A. M. Prince, W. Szmuness, S. J. Millian, D. S. David, *N. Engl. J. Med.* **284**, 1125 (1971).
8. D. J. Lang, *Arch. Gesamte Virusforsch.* **37**, 365 (1972).
9. R. N. Fine, C. M. Grushkin, M. Malekzadeh, H. T. Wright, Jr., *Arch. Surg.* **105**, 564 (1972); A. S. Coulson, Z. J. Lucas, M. Condy, R. Cohn, *West. J. Med.* **120**, 1 (1974).
10. D. Rifkind, N. Goodman, R. B. Hill, *Ann. Intern. Med.* **66**, 1116 (1967).
11. R. L. Simmons, R. Weil, III, M. B. Tallent, C. M. Kjellstrand, J. S. Najarian, *Transplant. Proc.* **2**, 419 (1970); D. S. Davis, S. J. Millian, J. C. Whitsell, G. H. Schwartz, R. R. Riggio, K. H. Stenzel, A. L. Rubin, *Ann. Surg.* **175**, 257 (1972); J. D. Briggs, M. C. Timbury, A. M. Paton, P. R. F. Bell, *Br. Med. J.* **4**, 520 (1972).
12. J. E. Osborn and D. L. Walker, *Infect. Immun.* **3**, 228 (1970).
13. L. B. Olding and M. B. A. Oldstone, *Fed. Proc.* **33**, 788 (1974); L. B. Olding, F. C. Jensen, M. B. A. Oldstone, *J. Exp. Med.* **141**, 561 (1975).
14. M. S. Hirsch, P. H. Black, G. S. Tracy, S. Leibowitz, R. S. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1914 (1970).
15. M. S. Hirsch, D. A. Ellis, P. H. Black, A. P. Monaco, M. L. Wood, *Science* **180**, 500 (1973).
16. M. S. Hirsch, S. M. Phillips, C. Solnik, P. H. Black, R. S. Schwartz, C. B. Carpenter, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1069 (1972); S. M. Phillips, M. S. Hirsch, J. André-Schwartz, C. Solnik, P. H. Black, R. S. Schwartz, J. P. Merrill, C. B. Carpenter, *Cell. Immunol.* **15**, 169 (1975).
17. R. J. Howard, J. Miller, J. S. Najarian, *Clin. Exp. Immunol.* **18**, 119 (1974).
18. Supported by NIH research grant 5R01-AI 11798. Preliminary results of this investigation were presented at the Fourteenth Annual Interscience Conference on Antimicrobial Agents and Chemotherapy in San Francisco, California, 11 to 13 September 1974.

17 March 1975; revised 15 May 1975

Increased Urinary Excretion of Cyclic Guanosine Monophosphate in Rats Bearing Morris Hepatoma 3924A

Abstract. *Urinary excretion of cyclic guanosine monophosphate (GMP) increased in rats bearing Morris hepatoma 3924A, and a correlation coefficient of .842 was observed comparing nucleotide excretion and tumor size. Irradiation of tumor or 5-fluorouracil administration delayed the increases in urinary cyclic GMP and tumor size. Surgical removal of tumors resulted in a rapid decline in cyclic GMP excretion to baseline levels. Cyclic adenosine monophosphate excretion was not altered by implantation, irradiation, or excision of tumor.*

The urinary excretion of cyclic guanosine monophosphate (GMP) increased significantly in rats bearing explants of Morris hepatoma 3924A. The increased excretion of cyclic GMP was diminished with local irradiation of tumor, 5-fluorouracil administration, or after tumor excision; however, the excretion of cyclic adenosine monophosphate (AMP) was not altered. These studies are of interest in view of a previous report describing increased levels of cyclic GMP in hepatomas in vivo (*1*). Rapidly growing hepatomas (3924A and 7288 ctc) contain very high concentrations of cyclic GMP (20- to 200-fold greater than normal liver or slower growing hepatomas). In other studies, the addition of exogenous cyclic GMP or its analogs to cell cultures increased DNA, RNA, and protein synthesis and cell proliferation (*2*). Rudland *et al.* (*3*) also described an activation of particulate guanylate cyclase from cell cultures of mouse fibroblasts with a fibroblast growth factor. In addition, Voorhees *et al.* (*4*) reported increases in cyclic GMP in rapidly growing epidermis from psoriatic lesions. In contrast, increased cyclic AMP in cell cultures has been associated with decreased proliferation and morphological changes resembling differentiated functions (*5*). However, studies with hepatomas in vivo have demonstrated increased cyclic AMP compared to liver (*1, 6*). Thus, the in vivo studies with cyclic GMP are consistent with cell culture studies and suggest that increased cyclic GMP is associated with proliferation. There have been no reports describing altered urinary excretion of cyclic nucleotides with tumors other than several hormone secreting tumors (*7*). Some of the observations reported here have been presented in abstract form (*8*).

Suspensions of Morris hepatoma 3924A were injected subcutaneously into the backs of female ACI rats weighing 150 to 180 g (*9*). Throughout the study rats were provided free access to laboratory chow