Growth of Parainfluenza Virus 1 in Isolated Perfused Rat Lungs[1] (35267)

P. Braun and J. O. Hendley
(Introduced by T. H. Weller)
John A. Hartford Foundation Organ Culture Unit, Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115

Growth of parainfluenza 1 virus (Sendai) in the intact human embryonic lung was reported recently from this laboratory (1), employing a new system of whole organ perfusion. Although the lungs degenerated progressively, virus multiplied when inoculated on the first to ninth days of perfusion. Because of the limited supply of human embryonic organs, variables in the perfusion system were explored by investigating growth of human parainfluenza virus in lungs from cesarean-derived, barrier-sustained, adult rats. This paper compares growth of virus in perfused and nonperfused tissues, and that obtained under varying conditions of perfusion.

Materials and Methods. Perfusion. The basic perfusion technique has been described (1). Tissue culture fluid, oxygenated by passage over a coil of gas-filled, silicone-rubber tubing, was pumped into the pulmonary artery of an isolated lung and returned to the oxygenator. The fluid was changed and sampled daily. Nonpulsatile flow in the arterial catheter was provided by a Holter Roller Pump (Model No. RDO 45 or RDO 75, Extracorporeal Specialties, Inc.). Pulsatile flow was obtained using a Sigmamotor infusion pump (Model TM 20-4, Sigmamotor, Inc., Middleport, N.Y., 14105), with Tygon pump tubing (R 3603, Norton Plastics and Synthetics Division, Akron, Ohio 44309). Flow rates for both the pulsative and nonpulsative systems were 2.5 $\pm$ 0.5 ml/min and the total fluid volume in the system was 60 ml.

The perfusion medium was Eagle's basal medium (BME diploid) with 20% heat inactivated fetal bovine serum (FBS). Other media tested included Trowell's T-8 (2) with 2% FBS, and McCoy's medium 5A with 30% FBS (3). The constituents were obtained from Grand Island Biological Co.

Oxygen tension in the perfusion fluid was varied by passing either 5% CO_2/95% oxygen or 5% CO_2/95% air through the oxygenator.

Animals. We used pathogen-free, albino, male rats, weighing 201–225 g (COBS rats, CD strain, Charles River Breeding Laboratories, Inc., Wilmington, Mass. 01887). The rats were anesthetsized with sodium pentabarbital, the intact lungs were removed aseptically, and the pulmonary arteries were cannulated with silicone rubber tubing (0.025 in o.d.).

Virus. We used the same strain of parainfluenza virus type 1 (Sendai/52) employed in earlier experiments (1). The infectivity titer ($TCID_{50}$) of pooled infected chick allantoic fluid was determined in primary human amnion cell cultures by hemadsorption of guinea pig erythrocytes. One ml of medium, containing 10^6 $TCID_{50}$ of Sendai virus, was inoculated into the pulmonary arterial cannula.

Hemagglutinin in the daily samples of medium was assayed by microtitration. Samples were diluted in phosphate buffered saline (PBS), pH 7.3, equal volumes of 0.5% guinea pig erythrocyte suspension in PBS were added, and tests were read after 1 hr at room temperature.

Results. Right and left lungs. Virus appeared in the perfusate 3 or 4 days after perfused rat lungs were inoculated with Sendai virus. The hemagglutinin response was similar to that from perfused human embryonic lungs (1), although the titers were lower. Preliminary experiments suggested that

[1] Supported by: A grant from the John A. Hartford Foundation; Research Grant AI-01023 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service; and by a grant from Parke, Davis and Company.

the two lungs from a single animal differed in their capacity to produce virus. In four experiments the right and left lungs from individual animals were inoculated and maintained under similar conditions. Hemagglutinin titers from the right lung were 4- to 8-fold greater than those from the left lung (Fig. 1). These findings are consistent with the fact that the rat right lung comprises four lobes and has approximately twice the volume of the one-lobed left lung. In all subsequent experiments we used paired homolateral lungs from animals of equal size.

Perfused versus nonperfused lung cultures. Hemagglutinin produced in a perfused rat

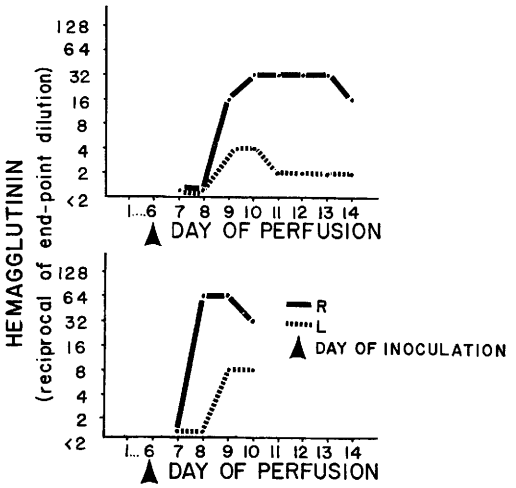

FIG. 1. Hemagglutinin titers of perfusates from right (R) and left (L) lungs in two representative experiments.

lung was compared with that produced by comparable quantities of lung tissue-fragments maintained in oxygenated media. One lung was cannulated and perfused via a nonpulsatile pump. A homolateral lung from a comparable animal was sliced into pieces about 1–2 mm thick, pooled, and bathed in perfusion medium circulated and oxygenated comparably with the perfused lung. A homolateral lung from a third animal was similarly sliced and the pieces were placed in a stoppered bottle containing previously oxygenated medium. The volumes of fluid were equal, and inoculation, fluid changes, and sampling were done in parallel on all three preparations. As shown in Fig. 2, hemagglu-

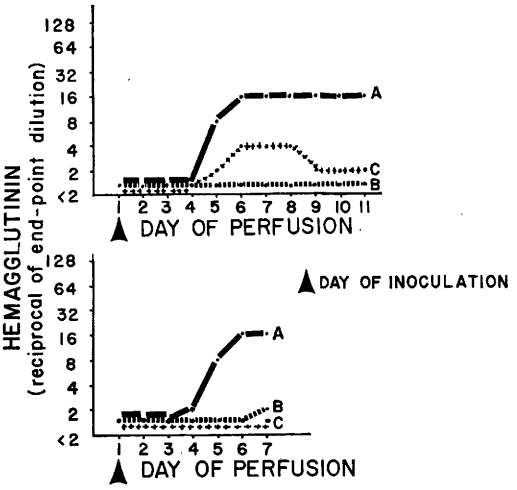

FIG. 2. Hemagglutinin titer of fluids from isolated perfused lung (A); fragments of one lung in circulating oxygenated medium (B); and fragments of lung in noncirculating preoxygenated medium (C) in two representative experiments.

tinin production from perfused whole lungs reached 1:16, titers from lung slices bathed in circulating oxygenated fluid reached $<1:2$ and 1:2, and titers from lung slices maintained in noncirculating oxygenated tissue culture fluid reached $<1:2$ and 1:4.

Oxygen tension. We assessed the effects of two different O_2 tensions on hemagglutinin production. The partial pressure of O_2 in the

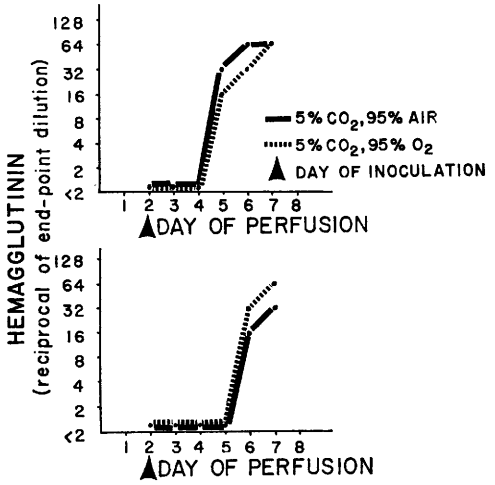

FIG. 3. Hemagglutinin titers of perfusates in two representative experiments comparing 5% CO_2/95% air with 5% CO_2/95% oxygen.

medium was 100–180 mm Hg when 5% CO_2/95% air was passed through the oxygenator, and 300–450 mm when 5% CO_2/95% O_2 was used. The pCO_2 was 10–20 mm Hg with either gas mixture. Neither the histologic appearance of the lungs nor the hemagglutinin titers (Fig. 3) varied with the gas mixture.

Type of pump. Titers of hemagglutinin were higher when pulsatile rather than linear flow was used (Fig. 4). This is in accord with reports based on morphologic studies that note the superiority of pulsatile flow perfusion (4).

Other variables. Hemagglutinin production was the same whether the lung was perfused at 0.5 or 2.5 ml/minute. The production of hemagglutinin was not enhanced by substituting either McCoy's or Trowell's medium for Eagle's BME/20% FBS. BME/20% FBS supplemented with 1% bovine Hb rapidly changed to dark brown when the oxygenator contained 5% CO_2/95% air passing through the oxygenator. Perfused whole lungs produced greater quantitites of hemagglutinin than did comparable amounts of fragments of lung tissue bathed in perfusion medium. The higher yields of hemagglutinin from the intact lung could be due to more efficient delivery of virus to susceptible cells, more efficient washout of virus from infected cells, or the persistence of larger numbers of cells capable of supporting viral replication.

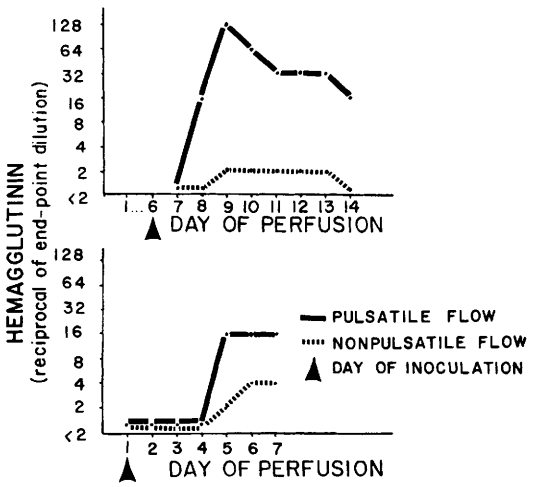

FIG. 4. Hemagglutinin titers of perfusates from lungs perfused by pulsatile and nonpulsatile flow in two representative experiments.

Some basic shortcomings of the perfusion system have not been resolved. Edema becomes grossly evident within a few hours of the start of perfusion, and degeneration of central parenchyma occurs within days. Nevertheless, the system permits the cultivation of a respiratory virus in a reproducible fashion and may be employed to explore further refinements in perfusion techniques.

Summary. Isolated, homolateral lungs of cesarean-derived barrier-sustained rats were maintained by a vascular perfusion with oxygenated tissue culture medium and supported the growth of the Sendai strain of parainfluenza type 1 virus.

Production of hemagglutinin by perfused lungs was greater than production by comparable quantities of sliced lung maintained in oxygenated perfusion medium. Homolateral lungs were required for controlled experiments of hemagglutinin production in perfused lungs. Pulsatile flow of perfusion fluid led to production of higher titers of hemagglutinin than flow at a constant rate. No differences were found when 5% CO_2/95% O_2 was compared with 5% CO_2/95% air and increase in virus production was not noted with several variations of the perfused medium.

We are indebted to Miss Harriet Cutler for her excellent technical assistance in all phases of the organ culture technique and for preparation of figures for publication.

1. Frothingham, T. E., Hendley, J. O., and Weller, T. H., Proc. Soc. Exp. Biol. Med. **133**, 1184 (1970).

2. Trowell, O. A., Exp. Cell. Res. **16**, 118 (1959).

3. Iwakata, S., and Grace, J. T., Jr., N.Y. J. Med. **64**, 2279 (1964)

4. Belzer, F. O., Ashby, B. S., May, R. E., and Dunphy, J. E., *in* "Organ Perfusion and Preservation" (J. C. Norman, ed.), p. 3. Appleton-Century-Crofts, New York (1968).

Received Sept. 21, 1970. P.S.E.B.M., 1971, Vol. 136.