Growth of Parainfluenza Virus in Perfused Human Embryonic Lung[1] (34651)

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Various obligate intracellular parasitic agents have been propagated in cultures of organized tissue fragments nourished by diffusion. The use of such cultures, for example to isolate rhinoviruses (1), or to cultivate poliomyelitis (2) or varicella virus (3) has yielded results superior to, or different from, those obtained with conventional monolayer cell cultures. These findings suggested that systems recently devised for maintaining whole organs by perfusion (4) might constructively be used in investigating infectious agents.

Technical practicalities have limited the duration of experiments on the behavior of infectious agents in perfused organs. Yoeli *et al.* (5), for example, studied pre-erythrocytic schizonts of *Plasmodium berghei* after 26 hr of perfusion of the liver of an infected tree rat. While normal physiologic function of perfused organs is still short, their useful experimental life may extend over many days or weeks (6). This report describes a relatively simple system for the prolonged perfusion of infected organs. Parainfluenza virus was selected to assess the utility of the system, and the growth of this virus in perfused intact human embryonic lungs is described.

Materials and Methods. Perfusion. The perfusion apparatus is depicted in Figs. 1 and 2. Two 50-ml syringe barrels were connected in a circuit of tubing, adaptors, and hypodermic needles. Perfusate was pumped through the arterial line (I and G) into the artery of the organ (H) held in a syringe barrel (A).

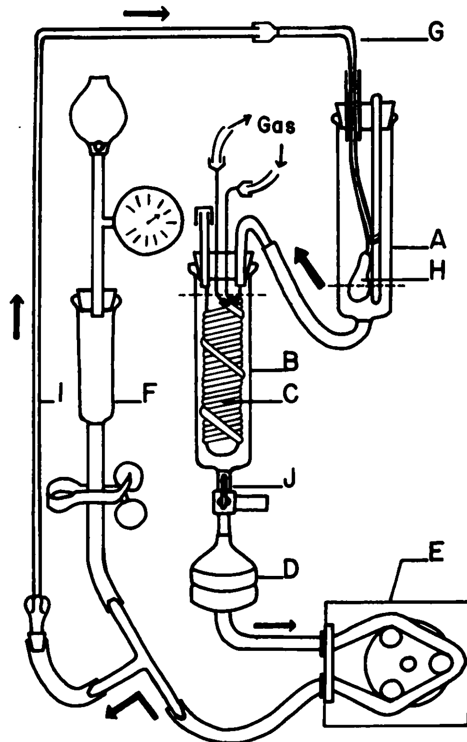

FIG. 1. Diagram of apparatus for perfusion of one organ. See text for explanation. Large arrows indicate direction of flow of perfusate; (---), fluid level in chambers.

After leaving the organ via the cut veins, the fluid was siphoned into the second syringe barrel (B) where it was exposed to an oxygenator (C) of the Folkman type (6). The fluid then flowed by gravity through a filter (D) to the pump (E). The arterial line (I) was connected to an assembly (F) which served to trap air bubbles and to measure arterial pressure.

Details of the apparatus and its application to lung perfusion follow. The oxygenator

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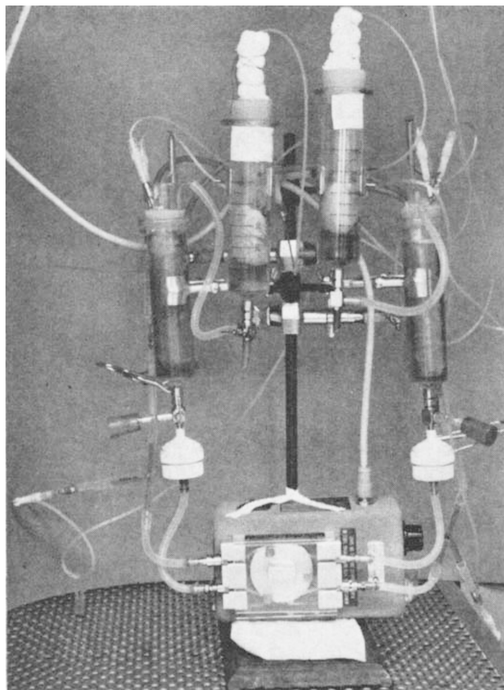

FIG. 2. Photograph of a pair of embryonic human lungs being perfused in two of the units illustrated diagrammatically in Fig. 1.

(C) consisted of a No. 6 silicone-rubber stopper pierced by two 20-gauge hypodermic needles, a 150 × 15-mm test tube, and two pieces of glass tubing. One needle was inserted into each end of a 14-ft length of "Silastic" medical-grade tubing (Dow Corning Corp.) of 0.025-in. internal diameter (i.d.). The tubing was wound around the test tube and secured with "Silastic" adhesive (silicone type A). Five percent CO_2 in oxygen was forced through the coil at an entering pressure of 5–20 mm Hg. The filter (D) was four layers of unweighted white silk (Joymar Scientific Inc.) held in a "Swinnex" casing (Millipore Corp.). The Holter roller pump (E) (Model No. RDO 45 or RDO 75, Extra Corporeal Medical Specialties, Inc.) provided nearly nonpulsatile flow simultaneously to two independent perfusion circuits. The external surface of the tubing in contact with the rollers was lubricated with castor oil. The pump was kept on its side to protect it from any leaking perfusate. The device for measuring arterial pressure (F) consisted of a 5-ml syringe barrel fitted with a rubber stopper pierced by a cotton filled glass tube in turn connected to a sphygmomanometer. This device (F) was clamped off from the arterial line except during measurements. The organ (H) was held in the organ chamber (A) with an assembly consisting of a No. 6 silicone-rubber stopper pierced by a short glass tube and a long glass rod. The arterial catheter (G) was "Silastic" tubing (0.02 to 0.040-in. i.d.); one end was attached by an 18- to 24-gauge needle to the arterial line (I) (Plexitron R 33 anesthesia extension set, Travenol Laboratories, Inc.). The other end passed through the glass tube in the stopper and was secured in the pulmonary artery with 3-0 silk thread. The arterial catheter and the appended organ were stabilized by attaching the catheter to the glass rod with thread. A gauze cuff was secured around the external opening of the glass tube and the entering arterial catheter. The remaining components consisted of "Silastic" tubing ⅛-in. i.d., and a variety of standard connectors and stopcocks (J). The apparatus was sterilized by autoclaving except for the arterial line (I) which was sterile as purchased.

Perfusate (60–80 ml) was circulated in the assembled apparatus for several hours before an organ was introduced. The perfusate was Eagle's basal medium (BME diploid) containing 20% fetal bovine serum (inactivated) obtained from Grand Island Biological Company. Perfusion was carried out in a conventional incubator at 36–37°. Before the organ was introduced, the apparatus was drained and refilled with fresh perfusate. Daily during perfusion (twice daily if acid production was excessive), the syringe barrels (A and B) were drained using the three-way stopcock (J) and were refilled with 50–70 ml of fresh perfusate. For virologic studies a few milliliters of the drained perfusate was stored at −70° and the remainder was kept at 4°; bacterial sterility was assessed by inoculation of thioglycollate broth.

Measurements. Measurements were made on perfusate withdrawn via stopcock J. pH and partial pressures of O_2 (pO_2) and CO_2 (pCO_2) were determined with conventional equipment (Radiometer, Copenhagen) de-

signed for analysis of blood. The usual values observed (except during the first few days when excessive acid was produced) were pH = 7.2–7.5, pO_2 = 250–450 mm Hg, and pCO_2= 10–20 mm Hg. The glucose content in spent perfusate was estimated with "Dextrostix" reagent strips (Ames Co.).

Arterial pressure was determined by releasing the clamp on the tube to the pressure-measuring device (F) and measuring the air pressure needed to hold the meniscus of the perfusate steady. Rate of flow was determined by clamping the siphon tube (between A and B) and observing the rate of filling of the organ chamber (A). In these experiments arterial pressure ranged from 20 to 100 mm Hg and flow was approximately 2.5 ml/min except as noted.

Virus. Parainfluenza virus type 1 (Sendai/52) was received from Dr. Keith Jensen in 1956. Two passages in chick embryo allantoic sac and three in primary rhesus monkey kidney or human amnion cell cultures had been made before the pool of infected allantoic fluid used in these experiments was prepared. Infectivity was determined in human embryonic kidney cell cultures (Microbiological Associates) maintained with BME. Titers were calculated as tissue culture infectious doses 50% ($TCID_{50}$) based on hemadsorption of guinea pig erythrocytes 7 to 9 days after inoculation of the cultures. Hemagglutinin titrations were done by conventional methods using guinea pig erythrocytes, and titers were expressed as the reciprocal of the initial dilution.

Organs. Lungs were obtained from human fetuses, about 13 to 20 weeks gestation, removed by hysterotomy for reasons other than infectious disease. In each experiment, paired lungs were perfused separately under similar conditions (Fig. 2). Parainfluenza virus was inoculated in 1-ml amounts intra-arterially; the inoculum per lung contained approximately 10^6 $TCID_{50}$.

Results. Four experiments were done involving perfusion of eight lungs.

Expt. 1. The findings in this experiment, shown in Fig. 3, also typify those in Expts. 2 and 3 (below). Two hr after inoculation, the infectivity titer of the perfusate was 10^3/0.2 ml, and the hemagglutinin titer was <2. A sharp rise in hemagglutinin titer in the perfusate occurred on the fourth day after virus inoculation, and there was a parallel increase in infectivity titer. The daily replacement of approximately five-sixths of the perfusion fluid *per se* produced a dilution of the inoculum of the order of 10^{-3} to 10^{-4} by day 6. Over the same period, the infectivity titer in the perfusate rose from 10^3/0.2 ml to 10^7/0.2 ml. Viral titers began gradually to decline after a peak on days 5 and 6. The perfusate from the uninoculated control lung showed no hemagglutinin activity and no infectious virus was detected. During the 24-hr period between replacement of perfusate, the glucose content usually decreased from the original 100–130 mg/100 ml to less than 45 mg/100 ml and frequently to undetectable levels. Beginning on day 5 however, glucose levels in the perfusate from the infected lung rose, suggesting decreasing viability of that organ.

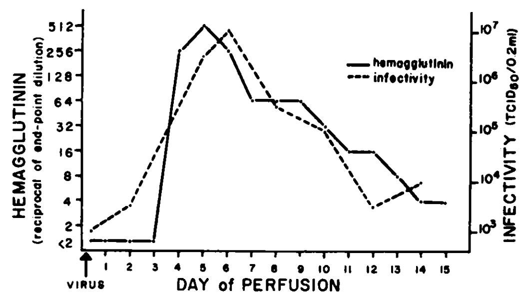

FIG. 3. Infectivity and hemagglutinin titers of perfusates from isolated human embryonic lung infected *in vitro* with parainfluenza virus type 1.

Expt. 2. A pair of lungs was perfused for 4 days before inoculation. On day 4, one lung was removed but perfusion of the empty chamber was continued. Virus was inoculated into both arterial catheters, one connected to the remaining lung and the other to the empty chamber. The rise in hemagglutinin and infectivity titers began on day 6 but occurred only in the circuit containing a lung.

Expt. 3. One lung was inoculated on the first day of perfusion and the pattern shown in Fig. 3 was repeated. The second lung, which had served as the control, was inoculated on the ninth day of perfusion. In this

lung, the rise in hemagglutinin and infectivity titers, and subsequent increase in residual glucose, appeared 3 days postinoculation, *i.e.* on the 12th day of perfusion.

Expt. 4. This experiment produced different results, perhaps because two variables were altered. First, the flow to each organ was reduced from 2.5 ml/min to about 0.5 ml/min by using pumping tubes of a smaller bore. In addition, the silk filter was inadvertently left out of the circuit. Virus was inoculated into one lung on the first day of perfusion and into the second lung on the eighth day. Hemagglutinin rise occurred in neither instance, although the infectivity titer in perfusate from the first lung increased from $<10^1$/0.2 ml to $10^{4.5}$/0.2 ml.

Gross changes in the lungs appeared during perfusion in all four experiments. A prominent lobular pattern became apparent in the edema-distended parenchyma. Large cavities in the parenchyma were sometimes present at the end of perfusion. For histological examination, one lung was fixed on the eighth day and three were fixed between the fifteenth and the twentieth days after inoculation of virus. There was extensive degeneration of the terminal air passages and alveolar tissue with better preservation of vessels and larger air passages. Specific viral effects, such as inclusion bodies, were not observed; this accords with findings at autopsy of patients with pneumonitis due to Sendai virus (7).

Discussion. Before concluding that Sendai virus had multiplied in the perfused lung, it was important to establish that viral growth was occurring in the organ itself and not in sloughed lung cells deposited in the perfusion system. In the second experiment, two lungs were perfused for 4 days to allow any free cells to become established. Following removal of one of the lungs, both circuits were inoculated with virus. The rise in hemagglutinin and infective virus titers occurred only in the system containing the organ. Thus it appeared that viral replication depended on the intact lung and not on any cells sloughed from it.

The general condition of a perfused organ may be judged in many ways. These include periodic assessments of glucose consumption and acid production, histological integrity, certain physiological activities, and the capacity to support growth of parasites such as malignant cells or a microorganism. In the system described here, viral growth was used as the chief criterion for judging the condition of the organ. The fact that viral multiplication was demonstrated in a lung inoculated as late as the ninth day of perfusion indicates that this system holds promise for the extended study of infectious agents. Current investigations are directed at evaluating variables to improve the system, and to attempts to cultivate organisms that cannot now be conveniently studied in the laboratory.

By convention, the term "organ culture" frequently is loosely and inappropriately applied to the cultivation of organized fragments of tissue maintained by diffusion of nutrients. Consideration might well be given to restricting use of the term to the maintenance of intact organs.

Summary. This report describes a relatively simple apparatus for the investigation of infectious agents propagated in intact, perfused, mammalian organs. Results of pilot experiments with parainfluenza virus in perfused isolated human embryonic lung preparations are summarized.

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