Device for Automatic Rapid Harvest of Roller Culture Supernatant Fluid
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Received for publication 27 February 1973

A machine has been designed to remove and replace supernatant fluids automatically from roller culture bottles at frequent intervals.

Morphological changes have been observed in Rous sarcoma virus (RSV) particles after they have budded from the cytoplasmic membrane (1). Rapid removal of supernatant fluids from virus-producing cells would allow biochemical analysis of changes which occur during extracellular maturation of RSV particles. An automatic device is desirable for virus collection, because these changes are likely to occur within a very short time, and harvesting the large quantities of virus necessary for biochemical analysis by manual techniques is laborious. The device should fulfill the following requirements: (i) complete removal of supernatant fluids from virus-producing cells at time intervals of 1 min or less, (ii) collection of supernatant fluids in chilled flasks to arrest viral maturation, (iii) automatic replacement of medium, and (iv) maintenance of virus-producing cells at physiological temperatures. The roller culture perfusion technique, which has been used to study the biological and biochemical properties of cells grown to high cell density (3), appeared ideally suited for this purpose. This paper reports the modification of a commercially available perfusion system (Model PF-4; New Brunswick Scientific Co. [NBS], New Brunswick, N.J.) which performs all of the desired operations.

The apparatus with which perfusion culture was performed was a modified NBS control apparatus (Fig. 1). Roller culture bottles of transformed chicken embryo fibroblasts were grown until confluent by conventional roller culture techniques before perfusion culture was started. The virus strain, medium, and roller culture conditions have been described (2; Smith and Bernstein, Appl. Microbiol., in press). A complete cycle of medium removal and replacement was performed as follows. (i) Rotation of roller culture bottles on the Rollacel (NBS RC-42) was stopped, (ii) the bottles were tilted forward with a cam device, (iii) a 5-s delay allowed medium to drain to the front of the bottles, (iv) medium was pumped out through a modified perfusion swivel cap (NBS M1007-1010) into a chilled collection flask, (v) medium was replaced through the modified perfusion swivel cap, (vi) the bottles were lowered to the horizontal position, and (vii) rotation of roller culture bottles on the Rollacel was resumed.

The following modifications on the New Brunswick perfusion system were necessary. On the perfusion swivel cap, the effluent line was shortened and bent downward to permit complete removal of medium from the front of the bottle (Fig. 2). The inlet tube was bent upward (Fig. 2) to permit gentle addition of fresh medium, because use of the unmodified inlet tube resulted in damage to the cell monolayer. On the cam device, a 1.27-cm shaft was inserted under the bottles, perpendicular to their long axis, and 25.4 cm from the back of the Rollacel. The shaft was turned by a motor equipped to deliver 4 rpm. Rotation of the shaft caused the attached cams (Fig. 3) to lift the rear of the bottles, tilting them forward at an angle of approximately 5° when the cams were stopped in an upright position. At the end of the cycle, the motor was reversed and the bottles were returned to the horizontal position. Eight electronic timers (Industrial Timers, Inc., Parsippany, N.J.) were required to allow the necessary operations (Fig. 4). The main timer regulated the length of time between medium removal-replacement cycles. Two timers were provided; a 3-h timer was used for intervals of 5 min to 3 h, and a 5-min timer was used for intervals of 5 min or less. The minimum collection time was 30 s, in which bottles were rotated for 12 s, and the balance of time was required to complete the collection cycle. The bottle-up timer regulated the length of time that the motor driving
the cam shaft was in operation. This timer was regulated to stop the cams at their maximum vertical elevation. The delay timer provided a 5-s delay to allow medium drainage to the front of the bottles after they were tilted forward, and it reversed the circuit to the motor driving the cam device. The pump-on timer activated a Kraft automatic pipettor (Model P300, Kraft Apparatus, Inc., Queens, N.Y.). It was necessary to plug the vent-inoculation port of the perfusion swivel cap with cotton, because a partial vacuum created in a sealed system by pumping medium led to a variable medium flow. Medium was pumped into prechilled collection vessels maintained either in an ice bath in the same room as the perfusion apparatus, or in an adjacent cold room maintained at 4 C (a hole was cut in the wall separating the rooms). The medium-add timer served the same function as in the unmodified system, namely, to activate a solenoid, which released pressure (provided by bottled nitrogen) on the automatic pinchcocks and allowed the addition of fresh medium by gravity flow. The amount of medium added to each bottle varied according to the frequency of collection. For example, 10 ml of medium was added per bottle during collection at 5-min intervals, and 25 ml of medium was added during collection at 2-h intervals. During extended perfusion cultures, the pH of the medium in the reservoir was readjusted daily by bubbling with carbon dioxide passed through a sterile, cotton-plugged pipette. The bottle-down timer lowered the bottles, and the reset timer prepared the electronic circuitry for the next cycle and started rotation of the Rollacel.

The machine has performed satisfactorily during numerous experiments for long-term collections of virus at frequent intervals. For example, roller culture bottles containing chicken embryo fibroblasts transformed with RSV were maintained on the machine for over 3 weeks, with collection of supernatant fluid every 2 h. The bottles were free of contamination during the entire period of collection, and no machine malfunction was encountered. Cell growth was vigorous under the perfusion conditions outlined. For example, a roller culture bottle maintained by perfusion culture yielded more than twice the number of cells obtained from a roller culture bottle maintained by daily medium changes (Fig. 5).

The device was designed for studying virus...
Fig. 3. Cam for lifting bottles. The cam was cut from 1.73-cm (11/16 inch) thick sheet of Lucite.

Fig. 4. Block diagram of the electronic circuit. All materials except timers were purchased locally.

particles recently budded from the cell surface. During the course of an investigation reported elsewhere (2), it was found that ribonucleic acid purified from RSV that was harvested at intervals of 2 h or less was more uniform in size than virus harvested at longer intervals (12 to 24 h). The manual harvesting of supernatant fluids every 2 h is tedious and can be done for only a portion of the day. Since the modified perfusion device allows automatic harvesting during the entire day, including weekends, the machine is currently being used to obtain large quantities of high-quality ribonucleic acid with relative ease.

The perfusion culture technique outlined could be used whenever sequential harvests of a virus, cell metabolite, or other extracellular material is desired.

This investigation was supported by Public Health Service grant no. CA12323 from the National Cancer Institute. We thank W. K. Joklik for support and encouragement. A patent application for this device is in preparation.

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