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Continuous Cultivation of Virus in Cell Suspensions by Use of the Lysostat

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ABSTRACT

GORI, GIO B. (Microbiological Associates, Inc., Bethesda, Md.). Continuous cultivation of viruses in cell suspensions by use of the lysostat. Appl. Microbiol. **13**:909–917. 1965.—Theoretical considerations illustrate the feasibility and characteristics of continuous virus cultivation in cell suspensions Physical dimensions of the virus fermentor (lysostat) in relation to optimal yields can be predetermined. Poliovirus type 1 was grown on Hela S-3-1 (Saltzman) cells in a single-stage, percolator-type lysostat, with a yield of 421 $TCID_{50}$ per cell. Adenovirus type 14 was grown on KB (Eagle) cells in a two-stage lysostat with a yield of 116 $TCID_{50}$ per cell.

The study of virus synthesis has been largely confined to stationary batch systems, where the growth variables are distorted by simultaneous phenomena of inactivation and interference. A dynamic continuous-growth system could mitigate this bias and support more immediate calculations of growth parameters. What is more, it would make possible a simple automation of virus production.

Since, at present, viruses are cultivated exclusively on living cells, a continuous virus fermentor requires a continuous supply of infectable cells. Suitable methods were described previously for continuous production of primary as well as heteroploid cell suspensions (Gori, 1964, 1965). The infectable cells enter the virus fermentor which, for features to be described, is called a lysostat. In the lysostat, the cells are infected and, after a certain maturation time, they are lysed by the virus, which is thus released in suspension ready for harvest.

As virus growth usually occurs at temperatures where thermal inactivation is rapid, it is desirable to withdraw the virus particles from the lysostat as soon as they are released from the cells. At the same time, no infected cells should leave the lysostat until lysed.

Under these conditions, the best fermentor appears to be of the feed-back piston flow type, with an overall residence time equal to the maturation time of the particular cell-virus system (Powell and Lowe, 1964). In practice such a fermentor is rather difficult to build, since the maturation time of most viruses is seldom less than 5×10^4 sec. This paper will show that a percolator-type, single-stage lysostat can provide an excellent compromise for cell-lysing viruses with high thermal-inactivation rates. A conventional two-stage lysostat can be efficiently used for thermostable viruses, and for viruses which do not lyse cells.

Theoretical considerations on a percolator-type, single-stage lysostat. A diagram of the lysostat is shown in Fig. 1. The source C (cell fermentor or automatic trypsinizer) releases a steady flow of infectable cells suspension to the lysostat, I, which is provided with a constant-level outlet and a filter, H, to prevent cells, but not the virus, from leaving the lysostat. The regulable volumetric pump, L, transfers medium from the reservoir, M, to the lysostat, thus eluting the virus suspension which is collected in the container, G.

If the volume of medium in the lysostat remains constant, medium entering the lysostat at an inflow rate F causes an equal outflow at the same rate. In perfect mixing conditions, a flow of medium through the lysostat washes out the medium and virus in the lysostat at a rate $D = F/V$, where F is the flow rate and V is the constant volume of medium in the lysostat. (From the volume V of medium in the lysostat, the volume of the cells in suspension should be substracted. However, this value is so small that in practice it can be neglected.)

Infected cells require a mean maturation time τ, after which they are lysed and release an average number β of infectious virus particles per each cell. In a population of random infected cells, the statistical rate of infectious virus synthesis per cell is, therefore:

$$\alpha = \frac{\beta}{\tau} \qquad (1)$$

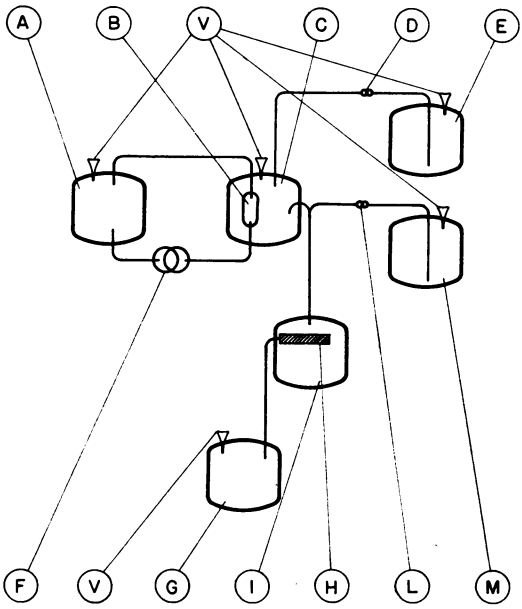

FIG. 1. *Single-stage percolator lysostat. (A) Dialyzing medium reservoir. (B) Dialyzing membrane. (C) Cell chemostat. (D) Cell chemostat flow-metering pump. (E) Chemostat feeding medium reservoir. (F) Dialyzing medium pump. (G) Virus-collecting reservoir. (H) Cell filter. (I) Lysostat. (L) Virus-eluting medium-metering pump. (M) Virus-eluting medium reservoir. (V) Air vents.*

The infectivity of most viruses is subjected to inactivation at a certain rate ι. Reactivation, interference, and absorption phenomena will also occur, but, for simplicity, they will not be considered in this analysis.

The infectable cell suspension enters the lysostat at a rate F_1 and, if it is assumed that once infected in the lysostat the cells do not multiply, then the concentration S of nonlysed cells in the lysostat follows the relation:

$$\frac{dS}{dt} = \frac{F_1 N}{V} - \frac{S}{\tau} \tag{2}$$

where N is the stationary concentration of cells in the suspension entering the lysostat, τ is the mean maturation time, and V is the volume of fluid in the lysostat. The relation 2 represents a system self-adjusting to stationary conditions where $dS/dt = 0$ and:

$$S = \frac{F_1 N \tau}{V} \tag{3}$$

The concentration S in the lysostat should be contained below toxic values; otherwise cell viability and virus yield are affected. If the maximal tolerable cell concentration is S_{max} then, from equation 3, the minimal volume of fluid in the lysostat is:

$$V_{min} = \frac{F_1 N \tau}{S_{max}} \tag{4}$$

The flow rate F_1 will cause a dilution rate $D_1 = F_1/V$ of the virus suspension. However, the dilution rate D of the virus suspension can be increased over the value of D_1 by introducing into the lysostat an eluting flow of medium at a rate F_2 which dilutes at an additional rate D_2. Therefore, the combined virus suspension dilution rate is:

$$D = D_1 + D_2 \tag{5}$$

Once the lysis is started, the relation of the concentration P of infectious virus particles in the lysostat is:

$$\frac{dP}{dt} = \alpha S - DP - \iota P \tag{6}$$

where t is the time, α is the rate of virus synthesis, S is the concentration of cells in the lysostat, D is the virus suspension dilution rate, and ι is the inactivation rate.

The concentration I of inactivated virus particles in the lysostat follows the relation:

$$\frac{dI}{dt} = \iota P - DI \tag{7}$$

where t is the time, ι is the inactivation rate, and D is the virus suspension dilution rate. In stationary conditions, it is $dI/dt = 0$ and:

$$I = \frac{\iota P}{D} \tag{8}$$

The efficiency q of infectious virus production can be expressed by the relation:

$$q = \frac{P}{I} \tag{9}$$

or, substituting from equation 8, $P/I = D/\iota$, it is:

$$q = \frac{D}{\iota} \tag{10}$$

Once the value of ι is known, it is, therefore, possible to insure a certain efficiency of infectious virus synthesis by selecting a proper value of $D > \iota$.

The minimal permissible volume of fluid in the lysostat is stated in equation 4 and, therefore,

the flow rate F_2 of eluting medium to be provided is defined as:

$$F_2 = DV - F_1 \quad (11)$$

where D is the virus dilution rate selected from equation 10, V is the volume of fluid in the lysostat as in equation 4, and F_1 is the flow rate of the cell suspension entering the lysostat.

The concentration P of infectious virus particles in the lysostat can now be calculated, solving equation 6 for $dP/dt = 0$:

$$P = \frac{\alpha S}{D + \iota} \quad (12)$$

where α is the virus synthesis rate, S is the concentration of cells in the lysostat, D is the virus dilution rate, and ι is the inactivation rate. The concentration P so calculated is the maximum obtainable under the selected parameters; higher concentrations can be obtained, but the synthesis of infectious virus particles will be less efficient, either because of cell death for population toxicity, if the value of S is increased, or because of a higher inactivation ratio P/I, if the value of D is decreased.

Once the system reaches stationary conditions, the value τ of the mean maturation time can be calculated, substituting $D_1 = F_1/V$ in equation 3:

$$\tau = \frac{S}{D_1 N} \quad (13)$$

where S is the stationary concentration of cells in the lysostat, D_1 is the dilution rate caused by the cell suspension inflow, and N is the cell concentration in the suspension entering the lysostat. The value of the virus synthesis rate α (see equation 1) can be calculated from equation 12 as:

$$\alpha = \frac{P\,(D + \iota)}{S} \quad (14)$$

where P is the concentration of infectious virus particles in the lysostat, D is the virus suspension dilution rate, ι is the inactivation rate, and S is the concentration of cells in the lysostat.

From equation 1 it is $\beta = \alpha\tau$ and, therefore, the average number β of infectious particles released by each cell can be calculated from equations 13 and 14 as:

$$\beta = \frac{P\,(D + \iota)}{D_1 N} \quad (15)$$

where P is the concentration of infectious virus particles in the lysostat, D is the virus dilution rate caused by the cell suspension inflow, and N is the cell concentration in the suspension entering the lysostat.

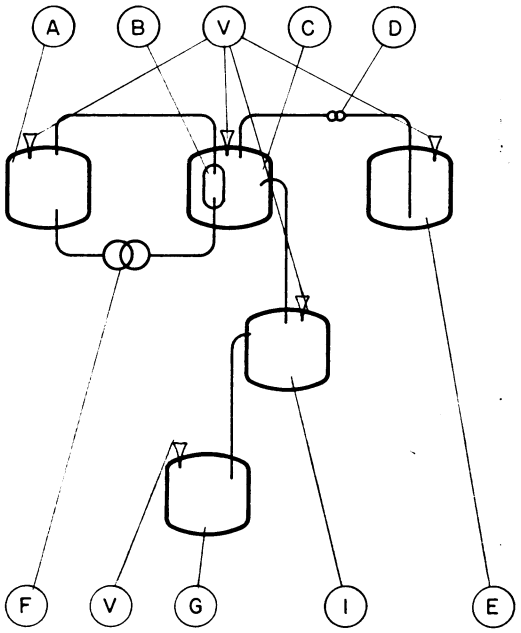

FIG. 2. *Two-stage lysostat. (A) Dialyzing medium reservoir. (B) Dialyzing membrane. (C) Cell chemostat. (D) Cell chemostat flow-metering pump. (E) Chemostat feeding medium reservoir. (F) Dialyzing medium pump. (G) Second-stage lysostat. (I) First-stage lysostat.*

Theoretical considerations on a two-stage lysostat. The two-stage lysostat diagram is given in Fig. 2. The source C (cell fermentor or automatic trypsinizer) releases a steady flow of infectable cells suspension to the first stage lysostat, I, which is provided with a constant-level outlet leading to the second-stage lysostat, G. In the first stage, the cells are infected and a fraction of them is lysed and releases virus in suspension. This virus infects the infectable cells which enter the first stage. A suspension of virus and infected cells flows from the first stage to the second stage where all cells are finally lysed by the virus.

The same considerations previously outlined for the dynamics of virus growth are valid. Under these considerations, the infectable cell suspension enters the lysostat at a rate F and each cell is lysed after a maturation time τ. Therefore, the concentration S of nonlysed cells in the lysostat follows the relation:

$$\frac{dS}{dt} = DN - DS - \frac{S}{\tau} \quad (16)$$

where $D = F/V$ is the dilution rate, N is the concentration of cells in the suspension entering the lysostat, and τ is the mean maturation time. For constant values of D, N, and τ, the relation 16 represents a system self-adjusting to stationary conditions where $dS/dt = 0$ and:

$$S = \frac{D N \tau}{D\tau + 1} \tag{17}$$

and:

$$\tau = \frac{S}{D (N - S)} \tag{18}$$

Once the infection is started, the relation of the concentration P_1, of infectious virus particles in the first stage is:

$$\frac{dP_1}{dt} = \alpha S - DP_1 - \iota P_1 \tag{19}$$

where α is the infectious virus synthesis rate, S is the concentration of nonlysed cells in the first-stage lysostat, D is the dilution rate, and ι is the inactivation rate. The relation 19 for constant values of α, S, ι, and D represents a system selfadjusting to stationary conditions where $dP_1/d\tau = 0$ and:

$$P_1 = \frac{\alpha S}{D + \iota} \tag{20}$$

from which:

$$\alpha = \frac{P_1 (D + \iota)}{S} \tag{21}$$

and substituting equations 1 and 18 in equation 21, it is:

$$\beta = \frac{P_1 (D + \iota)}{D(N - S)} \tag{22}$$

If equation 17 is substituted in equation 19, it is also:

$$\tau = \frac{\beta N}{P(D + \iota)} - \frac{1}{D} \tag{23}$$

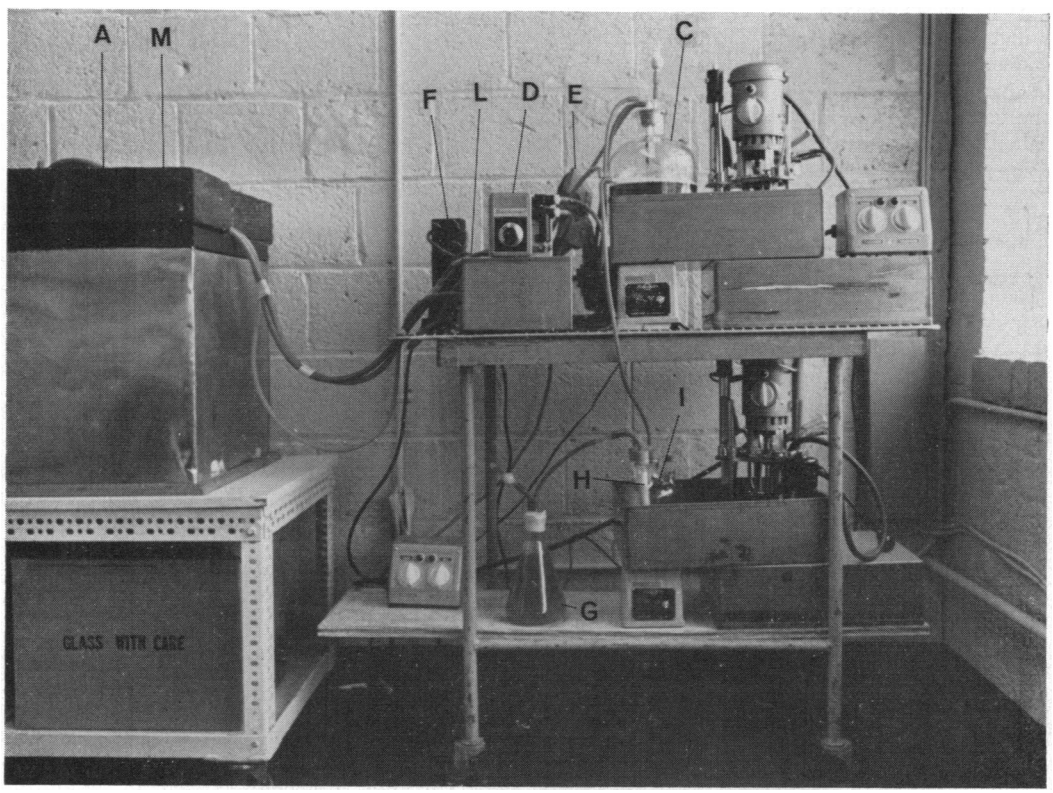

FIG. 3. *Single-stage percolator lysostat. (A) Dialyzing medium reservoir. (C) Cell chemostat. (D) Cell chemostat flow-metering pump. (E) Chemostat feeding medium reservoir. (F) Dialyzing medium pump. (G) Virus-collecting reservoir. (H) Cell filter. (I) Lysostat. (L) Virus-eluting medium-metering pump. (M) Virus-eluting medium reservoir.*

which allows a calculation of the mean maturation time τ without the bias of S.

For viruses not appreciably subjected to thermal inactivation, these relations become:

$$P_1 = \frac{\alpha S}{D} \quad (24)$$

and:

$$\alpha = \frac{P_1 D}{S} \quad (25)$$

and:

$$\beta = \frac{P_1}{N - S} \quad (26)$$

and:

$$\tau = \frac{\beta N}{PD} - \frac{1}{D} \quad (27)$$

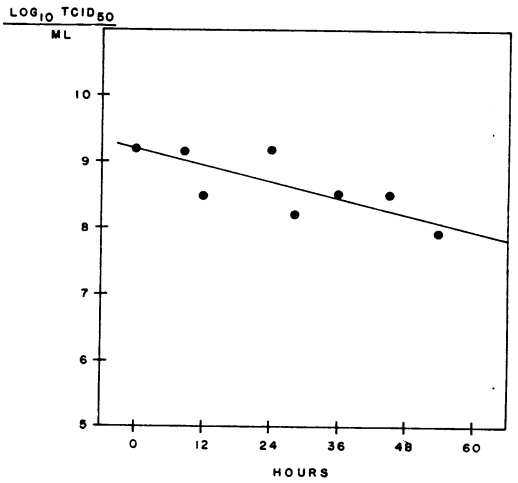

FIG. 5. *Poliovirus type 1, thermal-inactivation rate at 37 C, pH 7 to 7.3.*

The second-stage lysostat is a simple container where the overflow from the first stage accumulates. When the second stage is filled, it is removed and replaced with an empty vessel. After removal, each filled second-stage vessel is incubated to maturation for a time equal to the maturation time τ. For a thermostable virus, the theoretical final concentration P_2 in the second stage is:

$$P_2 = \beta N \quad (28)$$

from which the value of β can be easily calculated once complete maturation has been attained in the second stage.

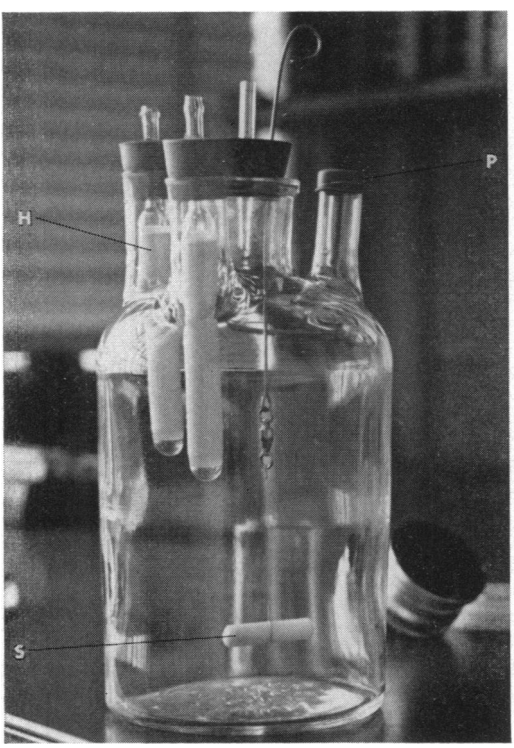

FIG. 4. *Single-stage, percolator lysostat. The lysostat proper. (H) Cell filter. (P) Sampling port. (S) Stirrer.*

MATERIALS AND METHODS

Cell chemostat. A 2-liter dialyzed-cell fermentor was previously described (Gori, 1965).

Percolator-type, single-stage lysostat. The lysostat is described in Fig. 1, 3, and 4. Pyrex glass containers of different volumes are used. The filter H is a 10-cm Corning no. 3500 sintered-glass candle, medium porosity, aseptically changed each time that free flow is impaired. The eluting medium pump L is a Beckman model 746 regulable from 0 to 5 ml per min. The eluting medium is injected through a connection at the immediate outlet of the cell chemostat; thus, the cells are washed down and are not retained in the tubings. The lysostat is immersed in a water bath at 37 C. Agitation is provided by a suspended magnetic bar, 5 cm long, at 100 rev/min. The collecting reservoir G is a 2-liter flask changed whenever needed. All tubing is surgical latex boiled in 5% sodium bicarbonate for 20 min and rinsed in distilled water. Vents are provided with cotton air filters. The entire unit is autoclaved at 120 C for 15 min.

Two-stage lysostat. The lysostat diagram is shown in Fig. 2. The first stage, I, is a Pyrex glass flask with an overflow at a constant volume of

approximately 1,320 ± 10 ml. The second-stage lysostat, G, is a 1,000-ml Pyrex glass flask. Agitation is provided in both stages by a suspended magnetic stirrer (5-cm bar) at 100 rev/min. Both stages are immersed in water baths at 37 C. Tubings, air vents, and autoclaving procedures are as described for the single-stage lysostat.

Cells. Hela S-3-1 (Saltzman) and KB (Eagle) cells were obtained from Microbiological Associates, Inc., Bethesda, Md.

Cell counts. Cell counts of nonlysed cells were performed in duplicate by staining one part of cell suspension with one part of a 1% Trypan blue isotonic saline solution. Cells were counted as nonlysed until totally stained by Trypan blue.

Media. Cell growth and dialysis medium was as described by Eagle (1959), supplemented with 10% inactivated calf serum, 100 units each per ml of penicillin and streptomycin, and 5 μg/ml of chlortetracycline. The virus-eluting and dialyzing medium was as described above, but no serum was added. Media used for cell growth and virus elution were sterilized by filtration through a cellulose acetate membrane with average pore diameter of 0.2 μ (Millipore Filter Corp., Bedford Mass.), after treatment in a continuous-flow ultraviolet irradiator (J. J. Dill Co., Kalamazoo, Mich.) at 1,000 ml per min. Media used for dialysis were not irradiated. All media were tested for cloning efficiency on HeLa S-3-1 (Saltzman) cells.

Viruses. Poliovirus type 1, Mahoney, used in these experiments was titrated in tube cultures of Rhesus kidney cells. Titrations were performed in duplicate with separate handling and 0.5 $\log_{10}$ dilutions.

Adenovirus type 14 was titrated in human embryonic kidney cells. Culture fluids were tested directly. Virus titers in the packed cells were obtained after freezing and thawing twice in one-tenth of the original volume. All titers were calculated by the method of Reed and Muench (1938).

Statistics. All mean values are arithmetical means of natural or logarithmic values as indicated.

RESULTS

Thermal-inactivation rates. The inactivation rate at 37 C and *p*H 7.0 to 7.2 for poliovirus type 1 is $\iota_{37} = 5.32 \times 10^{-6}$/sec, as calculated from data recorded in Fig. 5.

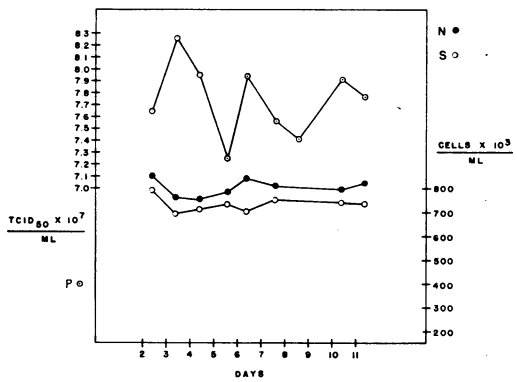

FIG. 6. *Growth of poliovirus type 1 in single-stage percolator lysostat. Symbols:* ⊙ *(P) = virus titer,* $TCID_{50} \times 10^7 \times ml^{-1}$; ● *(N) = cell concentration in cell chemostat, cells* $\times 10^{-3} \times ml^{-1}$; ○ *(S) = nonlysed cell concentration in the lysostat, cells* $\times 10^3 \times ml^{-1}$. *Lysostat volume, V = 930 ± 30 ml. For additional data, see Table 1, column A.*

TABLE 1. *Growth parameters at different lysostat volumes for continuous cultivation of poliovirus type 1*

Parameter	Dimension	A	B	C
ι = virus-inactivation rate	sec^{-1}	5.32×10^{-6}/sec	5.32×10^{-6}/sec	5.32×10^{-6}/sec
D = virus dilution rate	sec^{-1}	5.36×10^{-5}/sec	3.3×10^{-5}/sec	3.5×10^{-5}/sec
q = efficiency of infectious virus yield		10	6.2	6.59
V = volume of lysostat	ml	930 ml	2,015 ml	3,325 ml
F_1 = cell suspension inflow	ml sec^{-1}	1.66×10^{-2} ml/sec	1.66×10^{-2} ml/sec	1.66×10^{-2} ml/sec
F_2 = virus-eluting flow	ml sec^{-1}	3.33×10^{-2} ml/sec	5×10^{-2} ml/sec	1×10^{-1} ml/sec
N = chemostat cell concentration	ml^{-1}	8.2×10^5 cells/ml	7.5×10^5 cells/ml	7×10^5 cells/ml
S = lysostat cell concentration	ml^{-1}	7.3×10^5 cells/ml	4.09×10^5 cells/ml	2.31×10^5 cells/ml
P = infectious virus concentration in lysostat	ml^{-1}	7.47×10^7 $TCID_{50}$/ml	6.82×10^7 $TCID_{50}$/ml	3.52×10^7 $TCID_{50}$/ml
τ = virus mean maturation time	sec	5×10^4 sec	6.59×10^4 sec	6.61×10^4 sec
β = infectious virus yield per cell		301 $TCID_{50}$/cell	421 $TCID_{50}$/cell	406 $TCID_{50}$/cell
α = infectious virus synthesis rate	sec^{-1}	6.02×10^{-3}/sec	6.38×10^{-3}/sec	6.14×10^{-3}/sec

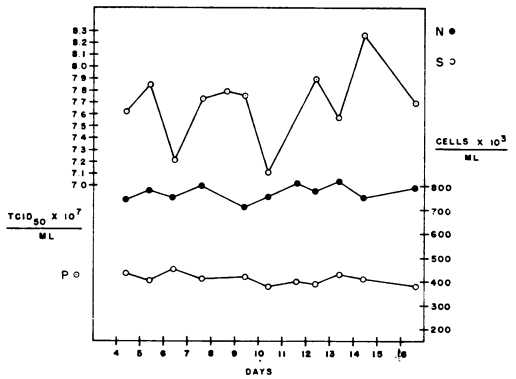

FIG. 7. *Growth of poliovirus type 1 in single-stage percolator lysostat. Symbols:* ⊙ *(P) = virus titer,* $TCID_{50} \times 10^7 \times ml^{-1}$; ● *(N) = cell concentration in cell chemostat, cells* $\times 10^{-3} \times ml^{-1}$; ○ *(S) = nonlysed cell concentration in the lysostat, cells* $\times 10^3 \times ml^{-1}$. *Lysostat volume, V = 2,015 ± 50 ml. For additional data, see Table 1, column B.*

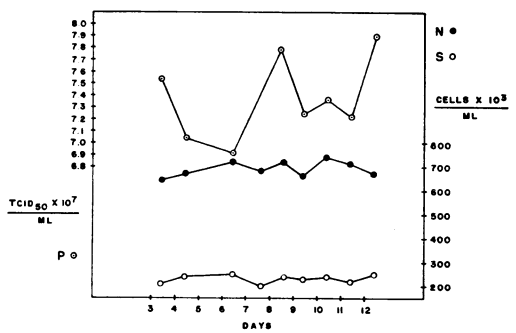

FIG. 8. *Growth of poliovirus type 1 in single-stage, percolator lysostat. Symbols:* ⊙ *(P) = virus titer,* $TCID_{50} \times 10^7 \times ml^{-1}$; ● *(N) = cell concentration in cell chemostat, cells* $\times 10^{-3} \times ml^{-1}$. ○ *(S) = nonlysed cell concentration in the lysostat, cells* $\times 10^3 \times ml^{-1}$. *Lysostat volume, V = 3,325 ± 50 ml. For additional data, see Table 1, column C.*

The thermal-inactivation rate for adenovirus type 14 is approximately $\iota_{37} = 3 \times 10^{-7}$/sec, as calculated from data published by Rafajko and Young (1964).

Continuous culture of poliovirus type 1. The operational parameters of the chemostat of Hela S-3-1 (Saltzman) cells are recorded in Table 1. According to Howes and Melnick (1957) and Ackermann, Loh, and Payne (1959), the maturation time for poliovirus is approximately $\tau = 8.10^4$ sec.

The maximal concentration of cells in the lysostat was tentatively set at 5×10^5 cells per milliliter, and, on the basis of equation 4, the minimal volume in the lysostat was calculated as $V = 2 \times 10^3$ ml.

The percolator-type, single-stage lysostat, described above, was used for the continuous cultivation of poliovirus type 1.

To test the validity of theoretical considerations, experiments were performed with lysostats of different volumes; the results are recorded in Table 1 and Fig. 6, 7, and 8.

Continuous culture of adenovirus type 14. The chemostat of KB (Eagle) cells was operated as indicated in Table 2. The two-stage lysostat described above was used for the continuous growth of adenovirus type 14. Experimental data are recorded in Fig. 10.

DISCUSSION

Percolator-type, single-stage lysostat. In Fig. 9, average values from several experiments indicate the variation of operational parameters in relation to the volume of the lysostat. If the volume of the lysostat is smaller than calculated by use of equation 4, toxic or antimetabolic conditions are probably established for the cells in the lysostat. These conditions result in loss of viable cells and, therefore, in decreased total virus yield and virus yield per cell. The same conditions are responsible for calculation of

TABLE 2. *Continuous cultivation of adenovirus type 14 growth parameters*

Parameter	Dimension	Data
ι = virus-inactivation rate	sec^{-1}	3×10^{-7}/sec
D = first-stage virus dilution rate	sec^{-1}	1.26×10^{-5}/sec
V = first-stage volume	ml	1,320 ml
F = first-stage flow	ml sec^{-1}	1.66×10^{-2} ml/sec
N = chemostat cell concentration	ml^{-1}	4.3×10^5 cells/ml
S = first-stage cell concentration	ml^{-1}	3.5×10^5 cells/ml
P_1 = first-stage infectious virus concentration	ml^{-1}	4.73×10^6 $TCID_{50}$/ml
P_2 = second-stage infectious virus concentration	ml^{-1}	5×10^7 $TCID_{50}$/ml
τ = virus mean maturation time	sec	7.59×10^5 sec
β = infectious virus yield per cell		116 $TCID_{50}$/cell
α = infectious virus synthesis rate	sec^{-1}	1.46×10^{-4}/sec

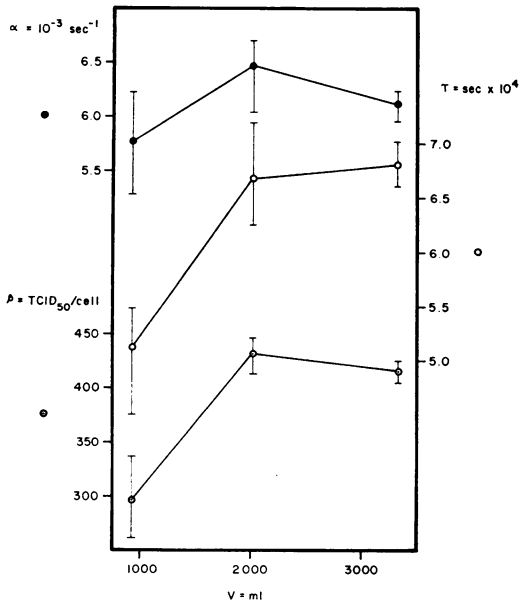

FIG. 9. *Growth of poliovirus type 1 in single-stage, percolator lysostats. Relation of growth parameters to lysostat volume. Symbols:* ● *(α) = virus synthesis rate,* × 10^{-3} × sec^{-1}; ⊙ *(β) = virus yield per cell,* TCID$_{50}$ *per cell;* ○ *(τ) = virus mean maturation time,* × 10^4 *sec. Five experiment averages at V = 930. Five experiment averages at V = 2,015. Three experiment averages at V = 3,325.*

maturation time values shorter than the real ones and, therefore, for biased calculations of the values of β and α. For the cell-virus system here described, it appears that cell concentrations in the order of 5 × 10^5 cells per milliliter are the maximum desirable in the lysostat. It may be possible to operate efficiently a lysostat at a cell concentration above this maximal value, provided that the lysostat is dialyzed. Perfusion is, of course, already achieved through the virus-eluting flow, but, apparently, either this is not capable of maintaining optimal cell viability at the flow rates employed, or some other causes of cell death intervene. It is also clear that if perfusion is to be effected with higher flow rates, then the virus titer will be decreased accordingly.

Two-stage lysostat. The usefulness of the two-stage lysostat is limited to the growth of viruses of very low heat sensitivity. The choice of the virus used in the experiments reported was perhaps unfortunate. Indeed, adenovirus type 14 appears to be rather thermostable, but the Trypan blue permeability of the infected cells has not a precise relation to the maturation of this intracellular inclusion-producing virus, and therefore it is difficult to calculate correct values of growth parameters. For instance, the calculation of the mean maturation time is based on the value of the nonlysed (Trypan blue-impermeable)-cell concentration in the lysostat, and, therefore, it is dependent on either the death of cells due to medium toxicity or on counts of unstained cells which should otherwise be considered as mature. Moreover, the real TCID$_{50}$ concentration is biased by a partition of free and cell-bound infectious virus particles, which does not follow a regular pattern, as indicated in Fig. 10. However unsuitable for calculation of growth parameters, the method appears satisfactory for continuous cultivation of this virus.

General conclusions. Experimental evidence indicates that the continuous production of viruses can be accomplished by relatively simple procedures. The optimal dimensions of the lysostat and the infectivity yield can be easily predetermined. Admittedly, the results here reported apply to small-sized operations and may face formidable problems in scaling up. The problem appears relatively simple for thermostable viruses. However, thermolabile viruses require the following special considerations if their infectivity is to be preserved and if maximal virus yield per cell is expected. (i) They should be removed from the lysostat as soon as the infectious units mature and, in any event, at a rate faster than the inactivation rate. (ii) Cells must stay in the lysostat until lysed, and most viruses require more than 5 × 10^4 sec for maturation in the infected cells. For this length of time, the cells cannot be kept physiologically normal above a certain critical concentration. If this critical concentration is exceeded, then toxic conditions for

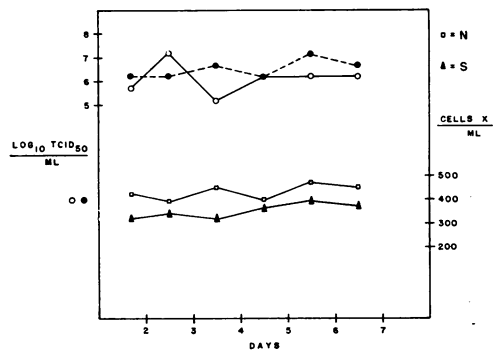

FIG. 10. *Growth of adenovirus type 14 in the two-stage lysostat. Symbols:* ○, *free;* ●, *cell-bound;* P = *virus titer in first-stage lysostat,* log_{10} TCID$_{50}$ × ml^{-1}; □, N = *cell concentration in cell chemostat, cells* × 10^3 × ml^{-1}; ▲, S = *nonlysed cell concentration in first-stage lysostat, cells* × 10^3 × ml^{-1}. *For additional data, see Table 2.*

the cells are established, and the virus yield per cell is decreased. (iii) Therefore, the maximal virus concentration obtainable under any condition of yield efficiency is limited. The concentration of virus, where necessary, should be performed by physiochemical means on the suspension produced by the lysostat. (iv) A major implication of these conditions is that high cell concentrations in the infectable cell source (cell chemostat) are not required or desirable.

From the reported experiments, it also appears that the calculations of virus growth parameters in continuous culture are contingent on the choice of monitoring criteria, such as the morphological definition of virus-cell maturation. These criteria may be easily defined for rapidly cell-destroying viruses (polio), but they may be deceiving for other viruses.

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