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Large-Scale Production and Concentration of Human Lymphoid Interferon

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A stable and predictable production system is described for pilot plant quantities (milligram) of human lymphoid interferon, using suspension culture of an African Burkitt's lymphoma derived cell line Namalva with induction by Newcastle disease virus, B-1 strain. Cell cultures were grown in impeller-driven 50-liter fermentors with dilution of the postinduction culture using serum-free medium. High levels of dissolved oxygen were necessary for optimum cell growth. A total of 4,207 liters of interferon culture was produced in a series of 116 fermentor runs. An average yield of 3.5 $\log_{10}$ international units of interferon per ml was realized before processing. Trichloroacetic acid was used to precipitate the interferon. An average of 3.35 $\log_{10}$ international units of interferon per ml was recovered in the final nonpurified product.

There is an increasing need for obtaining large quantities of interferon, particularly human interferon, for basic studies on chemical composition, sequencing, and clinical trials. There are reports (2, 3, 5, 8, 14) on mass propagation of tissue cells grown on solid substrates for interferon production. Only recently, Strander et al. (21), Zoon et al. (23), and Finter and Brigden (11) described both the necessity and possibility of large-scale production of human lymphoid interferon by using the Namalva cell suspension system for producing milligram quantities. Preliminary work on this system has been reported (4).

Interferons, generally species specific (13) in their range of activity, are a group of glycoproteins produced by a wide variety of tissue cells in vivo and in vitro after stimulation by a number of chemical or viral agents. Nearly all human clinical trials have been made with leukocyte interferon (20), although limited amounts of fibroblast interferon (7) have been available from time to time. The production costs and the availability of human donors have severely limited the amount of these interferons. Also, the growth and manipulation of cell cultures on solid substrates is cumbersome and limited, making it very costly to provide sufficient quantities for clinical use. Cell culture grown in suspension offers the dual advantage over the cultures grown on solid substrates of 10-fold-higher cell densities and a relative ease of increase in culture volumes to large production levels. The preliminary work reported (4) has been extended with the development of a reproducible large-scale suspension culture process for human lymphoid interferon at the 50-liter fermentor level.

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MATERIALS AND METHODS

Fermentors. The fermentors used in these studies were the same as those used by Klein et al. (17) for the production of mouse lymphoid cells. They were constructed by the New Brunswick Scientific Co., New Brunswick, N.J., as individual units consisting of two banks of four fermentors each. Each fermentor was individually outfitted with a mechanically sealed agitator shaft with three turbine impellers. Each fermentor console consisted of a recorder for pH, an agitator speed control, and a recorder-controller for temperature. Direct dissolved-oxygen (DO_2) utilization was determined by using a model 50 oxygen recorder attached to an autoclavable galvanic-type oxygen electrode mounted on the fermentor. The oxygen recorder was calibrated after the medium was incubated overnight at 37°C with a head pressure of 6 lb/in^2 and an rpm of 150. The gas sparger consisted of a single orifice immediately below the turbine-type impeller. Periodic air sparging at a low rate was used while maintaining an air overlay of 6 lb/in^2. This technique completely eliminated the need for antifoam agents.

Cell line characterization. The Namalva, or Namalwa, cell line was isolated from a clinical case of Burkitt's lymphoma (biopsy taken in Nairobi by S. Singh) by the staff of the Karolinska Institute (16) and taken back to Stockholm, Sweden, where it was established as a human lymphoma cell line. The master seed stock was obtained from G. Klein (Karolinska

Institute, Stockholm, Sweden). Cytogenetic studies show the cell line to have maintained a near-diploid modal chromosome number of 47, and it carries 13 different chromosomal abnormalities. Six of these abnormalities are characteristic of the line and are suited for use in monitoring the line during scale-up to production volumes. They are, in order of chromosome appearance: (i) marker-1, derived from chromosomes 1 and 10; (ii) a 1;3 translocation; (iii) monosomy-3; (iv) trisomy-7; (v) a 14q+ rearrangement derived from an 8;14 translocation; and (vi) a 15q deletion.

The 8;14 translocation, typical of Burkitt's lymphoma and occasionally seen in other lymphoid malignancies, was present in over 75% of the Namalva metaphases examined. No aberrations of 21q, which carries the antiviral gene locus controlling immune and classical interferon sensitivity (10), were detected.

Cell cultivation. Cell culture inoculum for the 50-liter fermentor was produced from frozen seed stocks maintained in liquid nitrogen vapor (−175°C). Primary and secondary (working stock) seed stocks were developed from the master stock for liquid nitrogen storage. Cells in each step of the build-up to fermentor production were progagated in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 40 μg of gentamicin per ml. Above the 6-liter culture volume stage, 25 mM HEPES buffer (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid) was added. Spinner flasks and/or serum bottles were spun at 100 rpm and/or shaken on the reciprocal shaker at 100 oscillations/min, respectively. The roller tube apparatus was operated at 1 rpm. Medium was pumped directly into the fermentor through a presterilized stainless-steel PALL filter housing consisting of one 1.2-μm cartridge and one 0.2-μm cartridge. FBS was added aseptically, using a peristaltic pump. The pH was adjusted to pH 7.2 with 2 N $NaHCO_3$ or 2 N HCl. The growth medium was incubated at 37°C overnight before cell inoculation. Incubation temperature for cell growth was at 37°C and was reduced to 35°C during virus absorption and interferon production. Namalva seed stocks, as well as fermentor cell cultures, were routinely monitored for mycoplasma with specific culture media. Viable cell counts were determined by using the trypan blue dye exclusion test.

Virus inoculum and induction procedure. A viral suspension in the form of infectious allantoic fluid derived from Newcastle disease virus, B-1 strain (NDV-B1), was used to induce interferon production in post-log-phase cell cultures at a concentration of 2.5 hemagglutination units (HAU) per 10^5 viable cells. The seed used was serially passaged in eggs and had a wide range of hemagglutination titers, ranging from 4,000 to 16,000 HAU/ml. Bulk lots of allantoic infected NDV-B1 were obtained from J. Campbell (National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health [NIH]) and used exclusively in this production system. Attempts to use crude commercial lots of allantoic infected NDV-B1 were unsuccessful, resulting in low yields of interferon. Undesirable by-products from mechanical separation of the infected allantoic fluid were found to be the underlying cause for the low interferon titer. After virus inoculation and absorption for 1 h at 35°C, the culture was diluted by using serum-free RPMI-1640 with L-glutamine and gentamicin as previously reported (23). The interferon production process was allowed to proceed for 40 to 44 h. Routinely, during the production period, samples were taken at 8-h intervals to study interferon kinetics. Samples were centrifuged, and supernatants were acidified (pH 2.0) with concentrated HCl and stored at 4°C for 24 h to inactivate the virus before the interferon assay (23).

Interferon assays. Interferon assays were conducted under contract by Biofluids Laboratory, Inc., Rockville, Md. The assay previously described (1) consists of a microtiter test using human foreskin fibroblast cells and vesicular stomatitis virus standardized against interferon reference standard (G-023-901-527, $10^{4.3}$ U/ml) maintained by H. Levy (National Institute of Allergy and Infectious Diseases, NIH). Titers are expressed as $\log_{10}$ of interferon dilution scoring virus cytopathic effects or as the reciprocal of the highest dilution of interferon demonstrating 50% or greater inhibition of cytopathic effect, which is considered the end point. The expression of interferon titer in a sample was determined from the average of triplicate determinations.

Processing procedure. The interferon was concentrated by trichloroacetic acid and processed after the induction cycle by the procedure of Bridgen et al. (4). The final precipitate was resuspended (0.5 g of precipitate/ml) with sterile distilled water, adjusted pH to 6.8, decanted into plastic blood packets, and stored at −70°C until delivered to NIH for further processing. All operations were performed at 4°C.

Statistical analysis. Regression analyses and other statistical analyses were performed on data by using the Biomedical Computer Program P-Series (9). Significant differences were based on obtaining a probability of less than 5% ($P < 0.05$) when statistical hypotheses were tested.

RESULTS AND DISCUSSION

The production of lymphoid interferon was found to be an interrelated process highly dependent on the success of each phase of the procedure for satisfactory yields. There were three main phases of the production of high-titer lymphoid interferon: (phase I) obtaining tissue culture in large volumes; (phase II) induction, dilution, and harvest of product; and (phase III) preparation and analysis of the bulk final precipitate.

Since large-scale production of lymphoid interferon from human cells grown in suspension is still in its infancy, the equipment and processes had to be adapted as the protocol developed. Data about both cell growth kinetics and interferon production kinetics in large volumes were incomplete, as were data on the environmental conditions necessary to obtain high-titer interferon. Recently, a cell dilution method suitable for both increased production volume and optimum interferon production was presented by Zoon et al. (23) and Bridgen et al. (4) and

incorporated into our process. We will discuss in some detail the production variables associated with each phase of the process as well as conditions necessary for large-scale production of human lymphoid interferon.

Phase I: large-volume tissue culture propagation. To provide uniformity and to maintain product integrity, each production lot of cell culture for interferon production originated from a single frozen ampoule of proven secondary seed stock. By carefully controlling the culture seeding density at each step, the cells from a single ampoule were scaled up to production levels (Fig. 1). This batch process consists of seven steps which run through 14 to 22 days of transfer and incubation.

(i) Inoculum build-up (steps 1 through 6). The tissue cell growth achieved during each of the six steps of the build-up before 50-liter fermentor inoculation was 1.5 to 2.0 doublings of the cell population. Doubling times of these six build-up steps ranged from 24 to 36 h, dependent upon vessel size and working volumes. With this build-up system, cell growth peaked at a cell density of about 2.5×10^6 to 3.5×10^6 viable cells/ml; cell cultures beyond logarithmic growth phase produced a lag period when inoculated into 50-liter fermentors independent of cell culture volume. Therefore, cell cultures before the peak (2×10^6 viable cells/ml) of the log phase were used as inocula for the 50-liter production fermentors (step 7) to avoid or reduce this stationary phase.

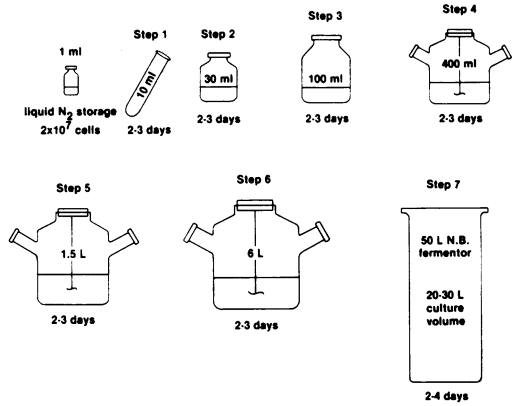

FIG. 1. *Production of cells for interferon production in a 50-liter New Brunswick fermentor. Step 1: 1 ml of frozen inoculum diluted in 9 ml of medium in a roller tube (25 by 150 mm). Step 2: 10-ml quantities of inoculum from step 1 added to 20 ml of medium in a 100-ml serum bottle. Step 3: 30 ml of inoculum from step 2 added to 70 ml of medium in a 500-ml serum bottle. Step 4: 100 ml of inoculum added to 300 ml of medium in a 500-ml Bellco spinner vessel. Step 5: 400 ml of inoculum from step 4 added to 1.1 liters of medium in a 3-liter Bellco spinner vessel. Step 6: 1,500 ml of inoculum from step 5 added to 4,500 ml of medium in a 20-liter Bellco spinner vessel. Step 7: each of eight 50-liter fermentors inoculated with separate 4- to 6-liter quantities of cells from the 20-liter Bellco spinner vessels. Cell numbers were sufficient to provide an inoculum in concentration greater than 4×10^5 viable cells/ml in 20- to 30-liter working volumes.*

(ii) Fifty-liter fermentor (step 7). In step 7 of the process, production volumes of Namalva cell culture were obtained. This step was initiated from spinner flask cell cultures of 4- to 6-liter working volume. The cell cultures were added to a known volume (16, 19, or 24 liters) of fresh medium. The total viable cells from the 4 liters equaled approximately 8.8×10^9 cells (22×10^5/ml $\times$ 4,000 ml), and when added to the fresh medium in the 50-liter fermentor, the cell density ranged from 3 to 5×10^5 viable cells/ml, which was considered the initial inoculum.

Since each fermentor was equipped with sterilizable pH and oxygen electrodes, the cell cultures were monitored for cell metabolism and growth by measuring pH and DO_2 utilization.

It was well known that sodium bicarbonate-buffered medium for cells grown in suspension was subject to rapid pH change. This problem was, for the most part, eliminated by adding HEPES buffer to RPMI-1640 production medium. If pH did show a drop below 6.8 during the cell fermentation period, 2 N NaOH was added to readjust the medium to pH 7.0 to 7.2. This was seldom necessary since adjustments of DO_2 levels, along with the HEPES buffer, were sufficient to maintain the pH at 6.8 to 7.2 for optimum cell growth.

The working volumes of production runs were deliberately varied so that cell growth characteristics in 20-, 25-, and 30-liter working volumes could be observed. It was also known that air sparging and agitation often results in foaming of tissue culture medium that can be detrimental to cell growth. The cell damage caused by too high an rpm level can occur over a prolonged time period. Therefore, an attempt was made to keep both of those parameters to a minimum. Since prediction of DO_2 requirements was not possible, the effects of oxygen on growth of Namalva cells had to be tested. It was first thought that the liquid-phase DO_2 in cell culture could be controlled by top (gas phase) aeration and a constant rpm level, maintaining a steady oxygen exchange rate created by the vortex from the impellers. This technique proved to be inadequate for DO_2 control, with cultures showing a substantial drop of DO_2 after the 1st day that

continued to zero DO_2 levels by the 3rd to 4th day of cell growth. Therefore, during the cell growth period, whenever the DO_2 level fell below 80% saturation, the cell cultures were sparged manually (1 liter of air/min) to regain 100% oxygen saturation. The viable cell densities and DO_2 utilization for a typical 50-liter fermentor are shown in Fig. 2A. Daily oxygen utilization is shown in Fig. 2B.

The cell culture volume played an important role in DO_2 exchange rate as well as subsequent cell growth rate and the cell densities achieved in the 50-liter fermentor. For a working volume of 20 liters, the doubling time was calculated to be, on the average, 31 h; for 25 liters, 39 h; and for 30 liters, 43 h. In view of the relationship of cell density levels achieved and grams-oxygen utilized, this observation could be expected. In cell cultures grown at suboptimum DO_2 levels with no sparging, the doubling time was observed to be 55 h.

High cell viability (90 to 95%) and density (2×10^6 to 4×10^6 cells/ml) were maintained in the DO_2-saturated cell cultures. In the nonreplenished cultures (with DO_2), a decline in cell growth and viability occurred as the cultures became more acid. Thus, low cell density levels occurred at low DO_2 levels, and high cell density occurred when DO_2 levels were high. The high cell density allows for increased level of cell dilution and an increase in interferon production.

Phase II: induction and cell dilution. Upon completion of the cell production in the 50-liter fermentors, the cell culture was induced by NDV-B1 (2.5 HAU/10^5 viable cells) at the apex of the post-log growth phase, which was found to be optimum for high-titer interferon production (Fig. 3A). Although cell cultures during the log growth phase produced interferon, the 4- to 5 day-old cultures were used exclusively for induction both to maintain high-titer interferon as well as to schedule a workable production process.

Routinely, the fermentor cultures were reduced in temperature to 35°C before induction with NDV-B1 virus, and the agitation rate was reduced to 75 rpm. Viral absorption time of 1 h was allowed in the culture vessel, after which time cell cultures were diluted with serum-free RPMI-1640 production medium and the rate was increased to 100 rpm. The volume of serum-free medium used to dilute the induced cell culture depended on the density of the culture. After dilution, the cell cultures were maintained at 35°C with an agitation speed of 100 rpm for an additional 39 to 43 h. They were then sampled

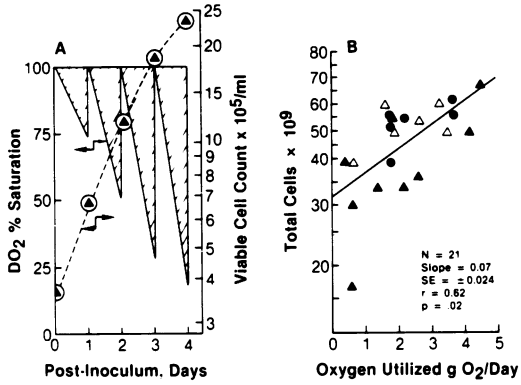

FIG. 2. *(A) DO_2, percent saturation, and growth of viable cells relative to postinoculum. The sparging necessary to maintain 100% DO_2 was translated to grams-oxygen (gO_2) quantities so that oxygen utilization could be correlated with cell growth. The total DO_2 in the system was the sum of the gO_2 in the headspace and the gO_2 in the liquid (nomogram for oxygen solubility in air-saturated fresh water, Orbisphere Corp., York, Me.). The gO_2 in the headspace was determined by integration of the following variables: (i) volume of air in headspace corrected to standard temperature and pressure; (ii) mole fraction of gO_2 in the headspace; (iii) fermentor temperature corrected for deviation from standard at 0°C; (iv) pressure adjustment to 6 lb/in^2 head pressure corrected for moisture content; and (v) molecular weight of oxygen in grams. The gO_2 in the cell culture was determined from the nomogram by the integration of fermentor temperature and barometric pressure converted to partial pressure, which was then related to fermentor temperature on the nomogram to determine O_2 (micrograms per gram) per liter of liquid. The total gO_2 for three working volumes of 20, 25, and 30 liters in the 50-liter fermentor are 10.54, 8.87, and 7.16, respectively. (B) Oxygen utilization per day (calculated from total depletion for all days divided by days of fermentation) relative to the cell density level of cell culture was measured in 20-liter (△), 25-liter (▲), and 30-liter (●) working volumes. The results were analyzed statistically by fitting a linear regression to the total cell yield at harvest (viable cells/milliliter × working volume). It was found that the total cell yield did in fact increase significantly with a proportional increase in gO_2/day of DO_2 utilized. Interpretation of the regression slope indicates that for each gO_2/day utilized, the total cell yield increases by 17%.*

and processed as described in Materials and Methods. It was found that interferon production and release was complete within 33 h postinduction, with no significant loss at 40 h. A typical cell growth pattern, induction, cell dilution, and interferon production in a 50-liter vessel (25-liter cell culture working volume) is illustrated in Fig. 3B. After induction and dilution

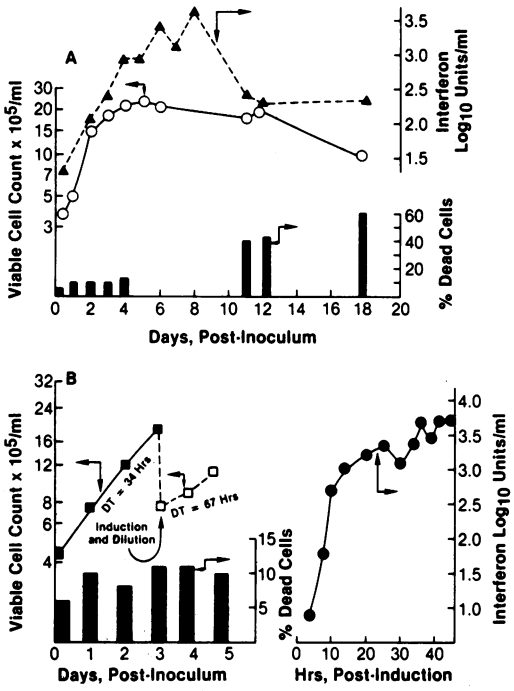

FIG. 3. *Growth of cell cultures and interferon titers in a 50-liter fermentor. (A) Interferon titers (determined on a 10-ml sample removed from fermentors and induced in the laboratory)* (▲) *during growth phase and* (○) *relative to days postinoculum. (B) Typical growth of cell cultures* (■), *growth after induction* (□), *and percentage of dead cells* (▮) *relative to time postinoculum; interferon kinetics* (●) *relative to time postinduction.*

(40 liters), the cells continued to grow or remained stable. In 72% of 36 production runs observed, cell growth continued at an average doubling time of 67 h, and the nonviable cells averaged about 12%. In the remaining static runs, nonviable cells increased slightly to approximately 20%. However, independent of cell growth, the interferon level obtained was not significantly affected. In contrast to these data, the cytogenetic analysis of the cells after induction and dilution showed the virus to inhibit cell mitosis and condense or pulverize the chromosomes. These results are in agreement with those of other investigators (6) in their studies with HeLa cells, and results cast doubt on the reliability of the dye exclusion test to measure true cell viability in a cell population after virus absorption has taken place and the cells are synthesizing interferon.

It was reported by Zoon et al. (23) that yields of interferon produced by diluted cell cultures were not related to FBS concentration (0 to 10%) and that dilution of cell culture with serum-free medium resulted in an increased production of interferon per cell. To study these effects on interferon production in the 50-liter system, data from 116 production runs were analyzed statistically. The interferon yield for this series of production runs ranged from 215 to 15,849 U/ml, and the postdilution viable cell densities ranged from 1.2×10^5 to 28.4×10^5 viable cells/ml. The postdilution concentration of FBS ranged from 2 to 10%.

To determine the relation of interferon yield per milliliter to percent FBS, a least-squares regression analysis (9) was used to analyze the 116 pairs of values. The interferon titer ($\log_{10}$ units per milliliter) was regressed on percent serum concentration, and the results confirmed that there was no significant relation between interferon yield per milliliter and percent FBS. Further analysis of the data from the 116 production runs showed that interferon yield was directly related to cell density (Fig. 4A), but that the yield per cell was inversely related to density, as shown in Fig. 4B and noted previously by Zoon et al. (23).

The interferon yield per milliliter (Y) increases relative to viable cells per milliliter (X) and can be expressed mathematically as follows:

$$Y = 1{,}175X^{0.44} \quad (1)$$

For each 10-fold increase in cell density, the interferon yield per milliliter increases 2.75-fold ($10^{0.44}$) (Fig. 4A). The relationship of Z (interferon units per cell) and X (viable cells per milliliter $\times 10^{-5}$) can be derived from equation (1) as follows. Since $Z = Y/X$, then $Y = Z \cdot X$. Now substituting $(Z \cdot X)$ for Y in equation (1), the following equation in Z and X is obtained:

$$Y = ZX = 1{,}175X^{0.44}$$

$$Z = 1{,}175X^{-0.56} \quad (2)$$

Equation (2) asserts that for a 10-fold decrease in X (cell density), a 3.63-fold ($10^{0.56}$) increase occurs in Z (interferon units per cell) (Fig. 4B). In the 116 production runs, the average $\log_{10}$ interferon units per milliliter ± 2 standard errors was recorded as 3.50 ± 0.07. Substituting these results in equation (1), it was determined that to obtain this yield of interferon would require X to be 9.5 (5.6 to 12.3) viable cells/ml $\times 10^{-5}$. This was equivalent to 950,000 (560,000 to 1,230,000 viable cells/ml), which was considered acceptable for obtaining adequate interferon yield.

Since the percent FBS does not affect interferon yields, and since low percentages of serum are desirable to facilitate purification, then cell densities as low as 600,000 viable cells/ml would

produce acceptable interferon yields with minimal FBS content.

Phase III: preparation, analysis, and storage stability of product. At the end of the induction period, the cell culture was processed for removal of infected cells and trichloroacetic acid precipitated, and the precipitate was collected with a Sharples continuous-flow centrifuge (4). The cell pellet was washed and retained for other experimental use. During processing of the acid precipitate, the supernatant was tested and found to contain <0.1% solids. After titration and resuspension of the collected acid precipitate, a sample was taken and diluted for assay to determine a material balance that in effect shows the percent removal and recovery of interferon for product evaluation. Since the concentrated trichloroacetic acid product was between 60× and 100× concentration, a direct assay of the product was not possible. To prove the reliability of our assay, assays were performed on a series of dilutions to determine whether the titer levels of the assay points follow the known dilution levels of the concentrate. Seventeen different trichloroacetic acid concentrates were diluted to 5×, 1×, 0.1×, and 0.01×, and interferon titers (measured in triplicate) were determined. The resulting average slope, i.e., the change in titers ($\log_{10}$ units) relative to change in log dilution, should be equal to unity (1.0). The average slope ± standard error from the concentrates was 0.975 ± 0.032. Thus, the observed slope was not significantly different from unity, and the assay procedure using 1× and 0.1× dilution (triplicate observations) for measuring interferon titer (activity) was considered accurate. The fact that each titer was based on triplicate observations presents an opportunity to obtain the precision with which a titer could be expressed. The standard deviation per assay observation was estimated to be ±0.31 $\log_{10}$ U/ml based on 461 df. The results compare favorably to the results found by Jameson et al. (15) of 0.17 to 0.40 $\log_{10}$ U/ml (depending on cell line) and by Viehauser (22) of 0.23 $\log_{10}$ U/ml.

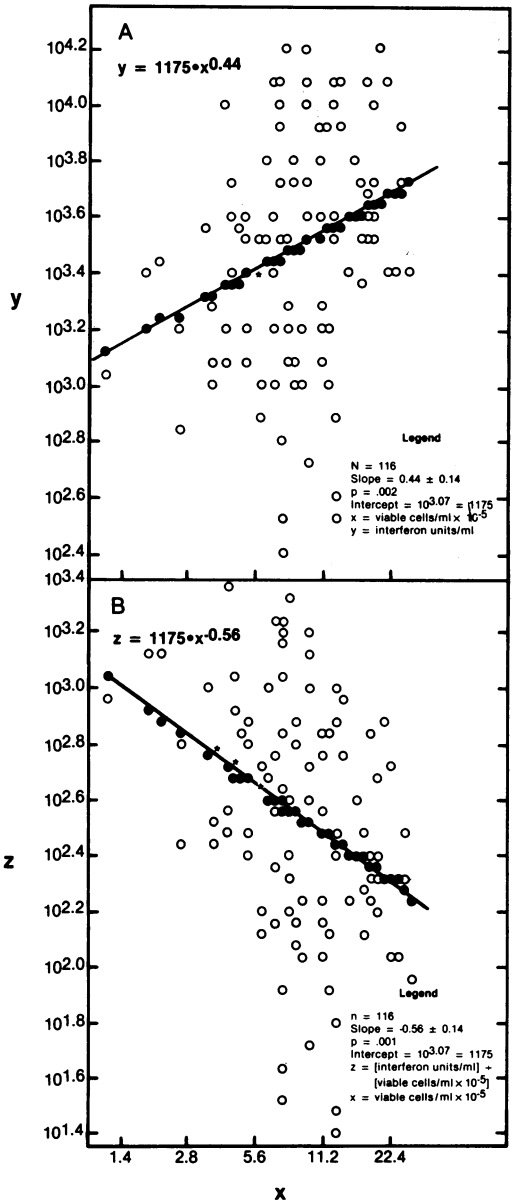

FIG. 4. *Interferon production efficiencies. (A) Interferon yields per milliliter relative to cell densities. (B) Interferon yield per milliliter per 10^5 viable cells relative to cell density. Symbols:* (○), *observed value,* (●) *predicted values;* (*) *when* ● *and* ○ *coincide.*

Stability of storage of the trichloroacetic acid product was unknown, so data on titer loss were obtained during holding periods of the product. Samples of 17 resuspended concentrates that had been stored for time periods ranging from 38 to 192 days at −20°C were assayed for titer loss. The average loss in titer was 0.0021 $\log_{10}$ U/ml per day. Thus, a concentrate stored at −20°C would require about 143 days to deteriorate 50% (i.e., loss of 0.30 $\log_{10}$ U/ml). All trichloroacetic bulk products were stored for no longer than 5 days before they were sent to NIH for further processing.

Production lots and material balance. The 116 runs in 50-liter fermentors are reported in 23 production lots. A lot consisted of two to eight fermentor runs. The results (Fig. 5) show interferon yield per lot expressed as titer per milliliter of diluted cell culture before and after processing. The after-processing titers were

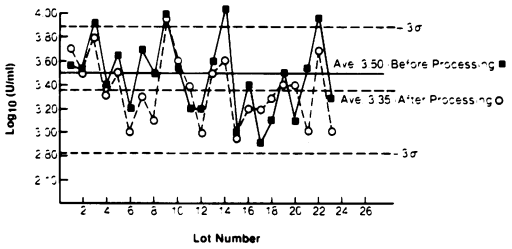

FIG. 5. *Interferon yield from 116 fermentor runs, 23 production lots. Total units after processing averaged 5.27 × 10^8 per lot, with a range of 0.7 to 16.0 × 10^8, from 1.2 kg of trichloroacetic acid percipitate per lot. Predilution cell cultures averaged 2.31 × 10^6 viable cells/ml and ranged from 0.5 × 10^6 to 3.92 × 10^6. After cell dilution, cell culture density averaged 1.13 × 10^5 viable cells/ml and ranged from 0.29 × 10^5 to 2.4 × 10^5. Total volume of cell culture per lot averaged 176 liters and ranged from 60 to 289 liters.*

based on triplicate assays of the concentrated trichloroacetic acid product resuspension diluted to culture concentration, i.e., 1×, so that data could be readily compared with titers obtained before processing. These yields averaged 3.50 and 3.35 $\log_{10}$ international units of interferon/ml before and after processing, respectively. The confidence limits associated with the assay itself are shown on the same figure for the after-processing average interferon yield. It was interesting and important to note that the majority of the production lots fell within the 3σ limits (where $\sigma = 0.31/\sqrt{3}$) of the assay variation, implying that the production system used here offers a reasonably stable and predictable method for obtaining milligram quantities of human lymphoid interferon.

The total volume for these 116 fermentations equaled 4,207 liters. Total interferon yield for these 116 fermentor runs was 1.21 × 10^{10} U. Assuming an interferon activity of 2 × 10^8 U/mg (18), this would provide for a cumulative production total greater than 60 mg of interferon.

For potential clinical application, there are three basic cell systems for interferon production: (i) primary leukocytes from buffy-coat cells obtained from human blood donors; (ii) lymphoid cells originally from biopsy of human tumors; and (iii) fibroblastic cells from human foreskins. All these cell types show diploid, or near-diploid, genotype. Similar or identical culture medium and supplements are used in the laboratory for production using all three cell types.

Fibroblastic cells are generally grown as monolayers in roller bottles, but recently success has been reported (19) with fibroblastic interferon production in suspension from microcarrier-grown cells. However, fibroblastic interferon is not identical to leukocyte interferon (12), and it appears to be unstable.

For human leukocyte interferon, 1 U of whole blood will yield 30 to 50 ml of culture fluid, with a total interferon yield of 1 × 10^6 to 2 × 10^6 U of crude interferon, or approximately 1 man-day/dose (2). Handling the number of units of blood necessary for practical clinical use would condemn leukocyte interferon to remain extremely expensive due to high labor costs even if sufficient donor blood were available. The current American Cancer Society clinical trials are utilizing leukocyte interferon at an approximate cost of \$50/10^6 U for short-term tests of \$50,000/patient (19).

Although much information has been reported over the last several months on the clinical availability of interferons, the availability and potential of routine production of uniform large quantities have been only in the developmental stages. We can definitely say that the uniform production of human lymphoid interferon at levels of 10^9 to 10^{10} U/run is feasible at significantly lower cost and depends only on the tank space available and equipment for product processing. It is worth noting that in large-volume suspension culture, the labor cost per unit is inversely related to volume. But perhaps equally important, the capability has been established at these facilities for the large-scale production of human lymphoid cells and by-products from these cells such as interferon and lymphokines, which are potentially useful in treatment and study of a large number of diseases. Recently, Zoon et al. (23) reported on the successful binding effects of lymphoid interferon produced by these cells to human leukocyte interferon antiserum. They reported that 95% of the lymphoid (activity) bound successfully to the antiserum. These results allow for the possible substitution of human lymphoid interferon for human leukocyte interferon in quantities needed to conduct laboratory studies and animal testing (primates) and eventual clinical studies for cancer chemotherapy and virus-related diseases.

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