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McAleer et al.

[11] **3,905,865**

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[54] **CELL AND VACCINE PRODUCTION**

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[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

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[21] Appl. No.: **474,279**

Related U.S. Application Data

[62] Division of Ser. No. 275,642, July 27, 1972, Pat. No. 3,839,155.

[52] **U.S. Cl.** **195/1.1**; 195/1.8; 195/127

[51] **Int. Cl.**[2] **C12B 1/00**

[58] **Field of Search** 195/1.7, 1.8, 127, 143, 195/1.1

[56] **References Cited**

UNITED STATES PATENTS

2,996,429	8/1961	Toulmin	195/143
3,407,120	10/1968	Weiss et al.	195/143
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Primary Examiner—Alvin E. Tanenholtz

[57] **ABSTRACT**

Use of tumbling in a rotating disc propagator to evenly distribute the cell slurry, obtain two side plating of cells, increase the cell concentration and recover the cells from the propagator in small volumes of medium, whereby the overall yield of cells and vaccines is significantly increased and at substantially reduced costs as compared to presently utilized procedures.

6 Claims, 6 Drawing Figures

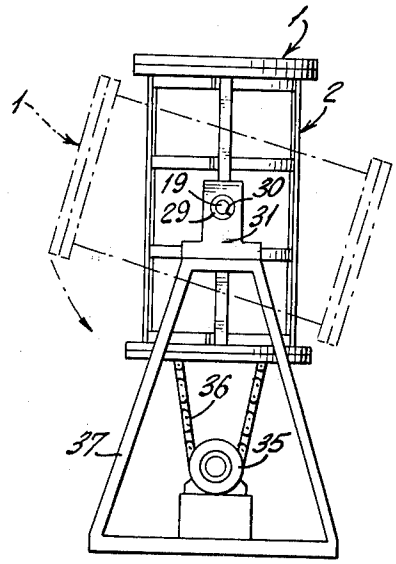


Fig. 1.

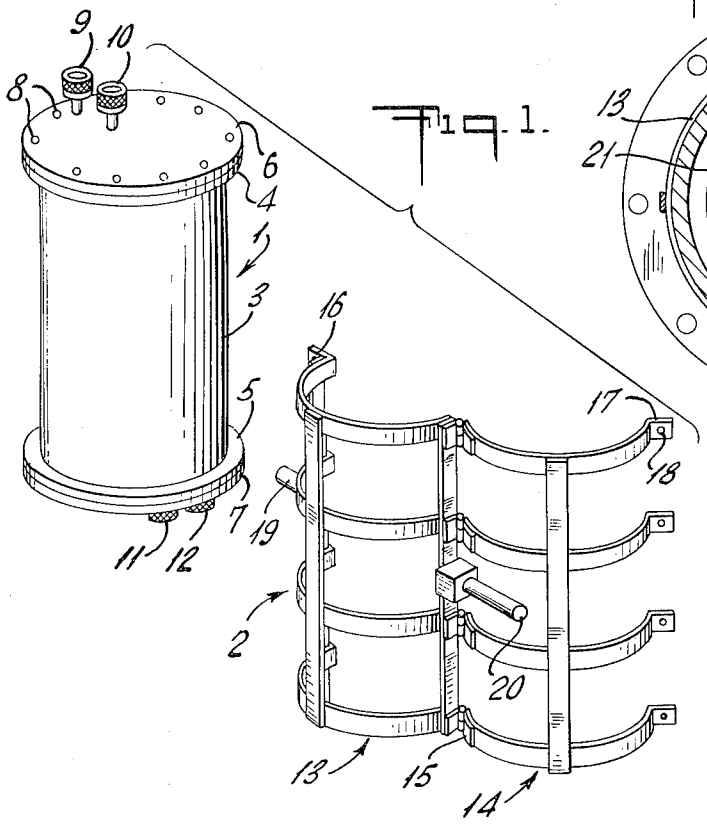


Fig. 3.

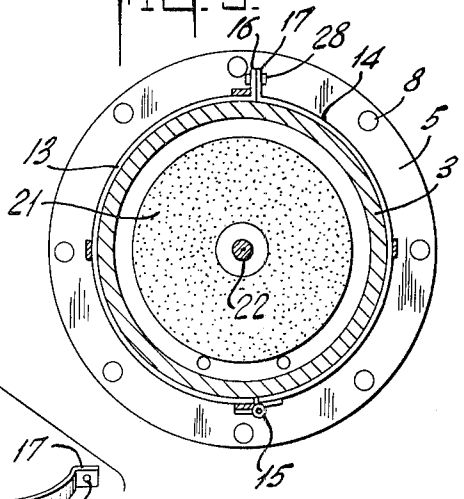


Fig. 2.

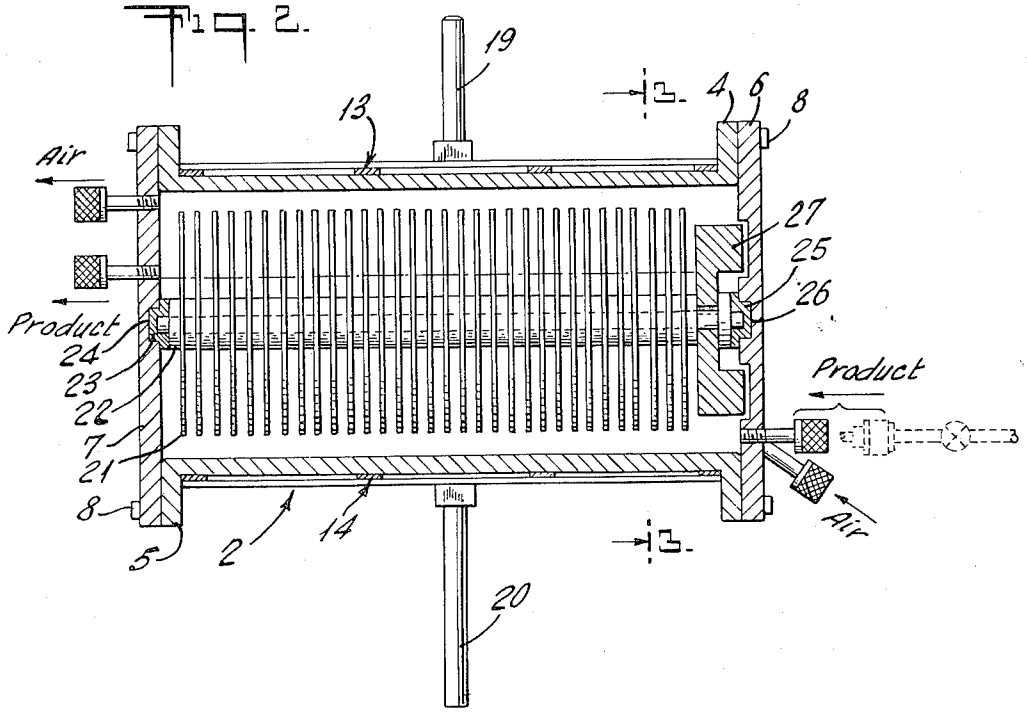


Fig. 4.

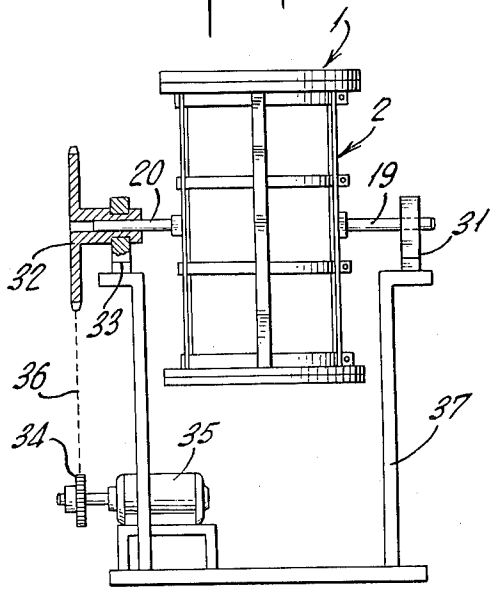


Fig. 5.

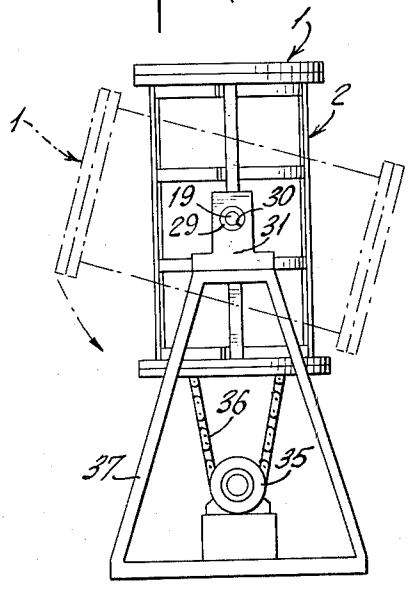
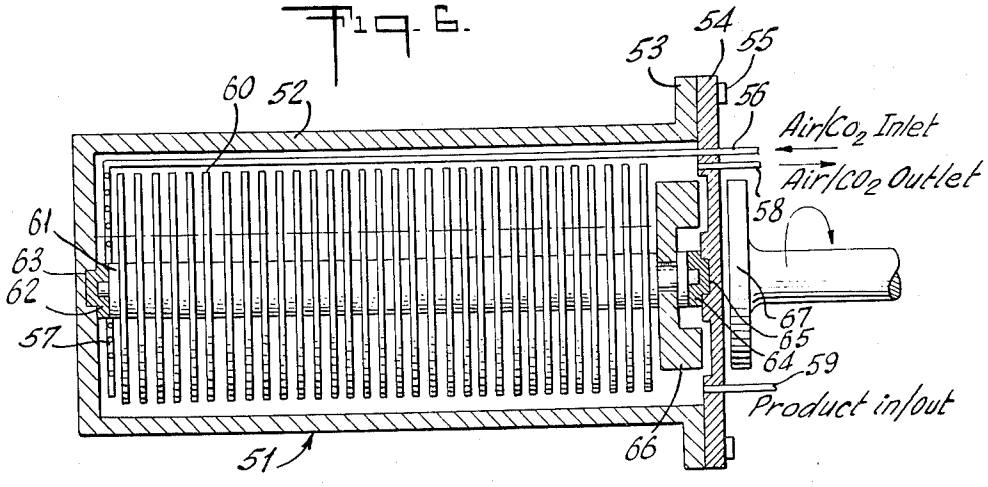


Fig. 6.



CELL AND VACCINE PRODUCTION

This is a division of application Ser. No. 275,642 filed July 27, 1972, now U.S. Pat. No. 3,839,155.

This invention relates to the production of cells and vaccines.

More particularly, this invention relates to the use of tumbling in rotating disc machines in order to produce cells and vaccines in substantially increased yields, thereby greatly reducing production costs.

Human and animal vaccines have been commercially produced by growing the desired virus in primary cells which must be grown on surfaces. Commercial processes were initially developed in Brockway bottles. These processes required the use of thousands of individual bottles to achieve the production of sufficient quantities of vaccine. The use of such a large number of bottles or production units is very time consuming and costly, and creates a substantial risk of contamination. As production techniques evolved, the original Brockway bottles were replaced by roller bottles which only slightly reduced the number of bottles and the handling problems associated therewith.

Some mass culture systems have been developed, such as the multiplate unit disclosed in U.S. Pat. No. 3,407,120 and the Biotec cylindrical rotating disc apparatus, but these units provide only minimal advantages over the original individual bottle system. In using a rotating disc apparatus, the greatest efficiency is obtained by minimizing the space between the plates, and growing cells on both sides of the plates. This however, creates a serious problem in obtaining a uniform distribution of cells and/or virus over both planar surfaces of the plates.

The present invention provides a method and device for overcoming the aforementioned disadvantages of the prior art procedures in which a cylindrical rotating disc apparatus is tumbled end over end at controlled speeds during the cell and vaccine production cycles, as for example during the cell plating, cell growth, cell washing, virus seeding, virus replication, and harvesting cycles of operation or during the addition of a chemical agent to the growth unit. For example, during the cell plating cycle, the propagator can be tumbled at a speed of from about 1 rev./5 min. to about 1 rev./15 min., preferably at a speed of about 1 rev./10 min., while during the virus seeding cycle, the propagator can be tumbled at a speed of from about 1 rev./3 min. to about 1 rev./8 min., preferably at a speed of 1 rev./5 min. and during the harvesting cycle at a speed of from about 30 rev./min. to about 90 rev./min., preferably at a speed of about 60 rev./min.

An advantage of the present invention is the ability to substantially increase the yield of cells and vaccines and thereby reduce the production costs.

A further advantage of the present invention is the ability to evenly distribute the cell slurry between the plates, thereby obtaining greater uniformity in the cell distribution and a higher yield of cells and vaccine.

A still further advantage of the present invention is the ability to obtain plating of cells on both sides of the plates from a single charge of cells without the necessity of a two-step procedure which requires two separate charges of cells, thereby increasing the yield of cells and reducing the risk of contamination and the costs associated therewith.

Another advantage of the present invention is that extremely low volumes of fluid can be used to bathe the cell sheet, and to recover the cells from the tank, thereby increasing the yield of cells or the titre/ml. of cell associated vaccine when compared to prior art procedures.

These and other advantages of the present invention will be readily understood by reference to the following detailed description when considered in conjunction with the accompanying drawings in which:

FIG. 1 is a perspective view of a typical multiplate propagator and holding means for the propagator employed in the present invention;

FIG. 2 is a cross sectional view of the propagator contained in the holding means;

FIG. 3 is a sectional view taken along the line 3—3 of FIG. 2;

FIG. 4 is a front elevational view of the tumbling device of the present invention;

FIG. 5 is a side elevational view of the tumbling device of the present invention; and

FIG. 6 is a sectional view of another propagator which is exemplary of those which can be utilized in accordance with the teachings of this invention.

Referring to the drawings, FIG. 1 discloses the multiplate propagator **1** and the holding means **2**. The propagator **1** includes a cylindrical stainless steel vessel **3** having top and bottom flanges **4** and **5** and a top plate **6** and a bottom plate **7**. Clamps **8** are used to seal plates **6** and **7** to flanges **4** and **5** respectively. Plates **6** and **7** also have fittings **9, 10, 11,** and **12** which connect to various lines when the propagator is in use in order to cycle air and fluids through the propagator. The holding means **2** also shown in FIG. **1** includes a cage like structure having two semi-tubular portions **13** and **14** which may be of an open or closed construction and are connected by hinges **15**. Portions **13** and **14** each have flanges **16** and **17** which have holes **18** through which pins or clamps may be inserted to close and latch the holding means **2** around the propagator **1**. The holding means **2** also has two cylindrical arms **19** and **20** extending from opposite sides of the mid-point of the holding means.

As illustrated in FIG. **2** the propagator **1** contains a series of titanium discs **21** mounted on a bar **22**, which supports the plates **7** in a separated state due to the presence of cylindrical spacers between each plate. One end of the bar is rotatably supported by a bearing **23** which is mounted in a recess **24** in the center of plate **7**. The other end of the bar **22** is rotatably supported by a bearing **25** which is mounted in a recess **26** in the center of plate **6**. A magnetic couple **27** is which is fixedly mounted on the bar **22** is engaged by magnetic drive means (not shown) in order to rotate the plates. The holding means **2** envelops the propagator and the edges of the semi-tubular members **13** and **14** about the edges of flanges **4** and **5** in order to hold the tank **1** in a fixed position.

FIG. **3** is a sectional view taken along line 3—3 of FIG. **2**, and shows the two semi-tubular portions **13** and **14** in the closed position and a pin **28** inserted in the holes **18** in flanges **16** and **17** to lock the holding means in the closed position around the cylindrical portion **3** of the tank **1**.

FIG. **4** is a front elevational view of the tumbling device of this invention and shows the propagator **1** contained in the holding means **2**. Arm **19** is surrounded by a bearing **29** rotatably mounted in hole **30** of supporting member **31** and arm **20** is fixedly mounted in

sprocket wheel 32 which in turn in rotatably mounted on support member 33. Wheel 32 is connected to sprocket wheel 34 of a motor 35 by a chain 36 in order to tumble the propagator 1. The motor 35 has a variable speed transmission in order to tumble the propagator at the desired speed. Support members 31 and 33 are positioned on stand 37.

FIG. 5 is a side elevational view of the tumbling device of FIG. 4 and illustrates the manner in which the propagator is tumbled end over end while positioned in the tumbling device.

Similarly in FIG. 6, there is disclosed another miltiplate propagator 51 which can be used in conjunction with the tumbling device of this invention. This propagator 51 comprises a cylindrical stainless steel vessel 52 having a flange 53 at one end thereof. Plate 54 is sealed to the flange 53 by clamps 55. An air-carbon dioxide mixture can be pumped into the vessel 52 from a reservoir (not shown) through a line 56 which extends along the length of the wall of the vessel 52 to the back of the vessel 52 where a portion of the line 56 extends along the back of the vessel. This portion of the line 56 has openings 57 which permit the egress of the air-carbon dioxide mixture. An outlet line 58 is also used to keep the air pressure within the vessel at a relatively constant level. Another line 59 may be used to supply medium, serum and other nutrients and to withdraw the expended medium and product. The plates 60 are mounted on a bar 61, which supports the plates 60 in a separated state due to the presence of cylindrical spacers between each plate. One end of the bar 61 is rotatably supported by a bearing 62 which is mounted in recess 63 in the bottom of the vessel 52. The other end of the bar 61 is also rotatably supported by a bearing 64 which is mounted in a recess 65 in plate 54. A magnetic couple 66 which is fixedly mounted on bar 61 is engaged by a driven magnet 67 to rotate the plates 60 through the medium 58 during the cell growth and virus infection stages of the production cycle.

The process and device of this invention may be used to produce viral vaccines such as mumps, measles, rubella, parainfluenza, Mareks and cells such as WI–38, chick embryo and duck embryo cells. Standard cells, sera and media may be used to produce the aforementioned vaccines. For example, primary cells such as chick embryo fibroblasts, green monkey kidney, bovine kidney, dog kidney or diploid cells such as WI–38 may be utilized as may standard sera such as fetal calf, calf, bovine, G-C-free newborn calf, α-gamma calf or α-gamma bovine and standard media such as Eagle's Basel Medium, Medium EBME, Medium 199, and Eagle's Minimum Essential Medium.

The invention will be better understood by reference to the following examples.

EXAMPLE 1

A rotating titanium disc propagator is charged with a mixture of 12 billion trypsinized chick embryo cells in Medium 199, 45 ml. 2.8 percent $NaHCO_3$/L and 10 percent fetal calf serum. The charged propagator is then locked into the tumbler and tumbled at 37°C. end over end at a speed of 1 rev./10 min. until two-side plating has occurred at which time the propagator is removed from the tumbler. The propagator is then positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution/8 minutes and air or a mixture of 95 percent air and 5 percent CO_2 is passed through the unit at a rate of 100 cc/minute until the cell growth cycle has been completed at which time the spent medium is discharged from the propagator, the propagator is washed with Hank's solution and charged with fresh Medium 199 containing 60 ml. 2.8 percent $NaHCO_3$/L 25 percent SPGA 4 millilitres of a mumps virus suspension which has a $-\log_{10}$ $TCID_{50}$/0.1 ml. of 3.6. The discs in the unit are again rotated at a speed of 1 revolution/8 minutes until there is no further increase in the concentration of virus in the supernatant fluids at which time the vaccine is harvested and frozen.

The yield of mumps vaccine when prepared by the above process is substantially higher than the yield obtained by using conventional procedures.

EXAMPLE 2

A rotating titanium disc propagator is charged with a mixture of 12 billion trypsinized duck embryo cells in Medium 199 containing 45 ml. $NaHCO_3$/L and 10 percent fetal calf serum. The charged propagator is then locked into the tumbler and tumbled at 37°C. end over end at the speed of 1 revolution/10 minutes until two side plating has occurred at which time the propagator is removed from the tumbler. The propagator is then positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution/8 minutes and air or a mixture of 95 percent air and 5 percent CO_2 is passed through the unit at a rate of 100 cc/minute until the cell growth cycle has been completed at which time the spent medium is discharged from the propagator. The propagator is then recharged with 2 litres of Medium 199 containing 60 ml. 2.8 percent $NaHCO_3$/L, 2 percent α -gamma calf serum and 170 ml. of a rubella virus suspension which has $-\log_{10}$ IND_{50}/0.1 ml. of 3.5 The propagator and contents are then locked into the tumbler and tumbled end over end at a speed of 1 revolution/15 minutes for 2 hours after which the propagator is removed from the tumbler and a further 6 litres of Medium 199 containing 60 ml. 2.8 percent $NaHCO_3$/L and 2 percent α-gamma calf serum is added to the propagator. The unit is then set so that the discs rotate at one revolution in 8 minutes with air or a mixture of air or 95 percent air and 5 percent CO_2 passing through it at 100 cc/minute at 37°C. When the infection process has been completed the spent medium is discharged and fresh Medium 199 containing 60 ml. 2.8 percent $NaHCO_3$/L 10 percent SPGA is added to the propagator. This medium in turn is discharged when the concentration of rubella virus has reached a maximum concentration.

This yield of rubella vaccine when prepared by the above process is substantially higher than the yield obtained by using conventional procedures.

EXAMPLE 3

A rotating titanium disc propagator is charged with a mixture of Medium 199, F 10 and tryptose phosphate broth with 5 percent fetal calf serum and 12×10^9 cells from trypsinized 12 day duck embryos and 14.4×10^6 PFU Marek THV. The charged propagator is then locked into the tumbler and tumbled at 37°C. end over end at a speed of 1 revolution/20 minutes until two side

plating has occurred at which time the propagator is removed from the tumbler. The propagator is then positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution/8 minutes and air or a mixture of 95 percent air and 5 percent CO_2 is passed through the propagator at a flow rate of 100 cc/minute. The pH is adjusted from time to time with 7.5 percent $NaHCO_3$ so that it remains within the limits of pH 6.8–7.7. Also glucose is added to the system periodically so that at no time should the glucose concentration go outside the limits of 15–100 mg/100 ml. On the 6th day after plating the spent medium is discharged and 6 litres of KCL-citrate/Trypsin is transferred into the propagator. The discs are rotated two complete revolutions and then the KCl-citrate/Trypsin solution is voided. The propagator is then locked into the tumbler and held with the plane of the discs in the horizontal axis for 5 minutes after which it is rotated about its long axis 90° so that the plane of the discs is in the vertical axis. While in this position 1.5 L of Eagle's Basel Medium containing 15 percent fetal calf serum is pumped into the propagator. The propagator and its contents are then rotated end over end at a speed of 1 revolution/second for 10 minutes. Following this tumbling the contents are discharged and assayed for Marek TVH.

The yield of Marek's vaccine when prepared by the above process is substantially higher than the yield obtained by using conventional procedures.

EXAMPLE 4

A rotating titanium disc propagator is charged with a mixture of 300×10^6 WI-38 cells in Medium EBME containing 10 percent fetal calf serum and 10 ml. of glutamine/L. The propagator and its contents are then held with the plane of the plates in the horizontal axis at 37°C until plating has been achieved. The propagator is then positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution/5 minutes and air or a mixture of 5 percent CO_2 and 95 percent air is passed through the unit at a rate of 100 cc/minute. Twenty-four hours later the medium is discharged from the machine and the unit is refilled with an equal volume of fresh Medium EBME containing 5 percent fetal calf serum and 10 ml. glutamine/L. After a further 48 hours in the rotating and gassing mode, the cell suspension is harvested from the unit. For this operation the unit is voided of spent medium and is then half filled with a solution containing trypsin. The plates are rotated through the trypsin solution so that all parts of each plate contact the solution. The trypsin is then voided. After waiting 5 minutes, 1 L of Medium EBME containing 15 percent fetal calf serum is pumped into the propagator and the whole unit is then tumbled end over end at a speed of 1 rev./sec. for 10 minutes, after which the cell suspension is discharged and collected. By use of the above procedure there is obtained an increase of cell yield of 300 percent over the conventional procedure.

EXAMPLE 5

A rotating disc propagator is charged with a mixture of 3.0 billion trypsinized chick embryo cells, Medium 199, 45 ml. 2.8 percent $NaHCO_3$/L and 5 percent fetal calf serum. The propagator is held in the vertical position at a temperature of 37°C and plating is effected. After 3 hours the fluid in the propagator is discharged and a further 3.0 billion trypsinized chick embryo cells are added to the fluid. After mixing the fresh suspension is transferred back into the propagator which is held in the opposite vertical position to the first plating at a temperature of 37°C in order to effect plating on the second side of the discs. When this has been accomplished, the propagator is positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution in 5 minutes and air or a mixture of 5 percent CO_2 and 95 percent air is passed through the propagator at a rate of 100 cc/min. When the cells have reached the confluent state or the growth has ceased, the cells may be harvested.

The medium in the propagator is discharged and the propagator is filled up to the halfway mark with a solution containing trypsin. The discs are rotated twice so that all parts of the disc become wetted with the trypsin.

The trypsin solution is then discharged and the unit is held with the plane of the plates in the horizontal axis for 5 minutes. The propagator is then charged with 1 L of fresh Medium 199 and 45 ml. 2.8 percent $NaHCO_3$/L and is tumbled end over end at a speed of 1 rev./min. for 10 minutes after which the cell suspension is discharged.

In this way it was possible to prepare with great efficiency and economy large quantities of chick embryo cells.

EXAMPLE 6

A rotating disc propagator is charged with a mixture of 3.0 billion trypsinized duck embryo cells, Medium 199, F 10 5 percent fetal calf serum and 30 ml. 2.8 percent $NaHCO_3$/L. The propagator is held in the vertical position at a temperature of 37°C and plating is effected. After 3 hours the fluid in the propagator is discharged and a further 3.0 billion trypsinized duck embryo cells are added to the fluid. After mixing the fresh suspension is transferred back into the propagator which is held in the opposite vertical position to the first plating at a temperature of 37°C in order to effect plating on the second side of the discs. When this has been accomplished, the propagator is positioned so that the plane of the discs is in the vertical axis and a portion of the medium and serum is discharged until the unit is about half full. The discs are then rotated at a speed of 1 revolution in 5 minutes and air or a mixture of 5 percent CO_2 and 95 percent air is passed through the propagator at a rate of 100 cc/min. When the cells have reached the confluent state or the growth has ceased, the cells may be harvested.

The medium in the propagator is discharged and the propagator is filled up to the halfway mark with a solution containing trypsin. The discs are rotated twice so that all parts of the disc become wetted with the trypsin.

The trypsin solution is then discharged and the unit is held with the plane of the plates in the horizontal axis for 5 minutes. The propagator is then charged with 1 L of fresh Medium 199, F10 5 percent fetal calf serum, 30 ml. 2.8 percent $NaHCO_3$/L and is tumbled end over

end at a speed of 1 rev./sec. for 10 minutes after which the cell suspension is discharged.

In this way it was possible to prepare with great efficiency and economy large quantities of duck embryo cells.

What is claimed is:

1. In a process for propagating cells or a virus wherein a liquid culture medium and culture cells are added to a propagator vessel capable of both rotatable and tumbling motion, the vessel containing a plurality of impermeable separated plates rotatively mounted on a shaft, the plates capable of supporting growth of cells on both sides of the plates, wherein culture cells are plated on the plates, wherein the medium optionally is seeded with a virus, and wherein the propagator cells or virus are harvested, the improvement which comprises tumbling the propagator vessel end over end at controlled speeds during at least one of the cell plating cycle or the cell harvesting cycle or the virus seeding cycle.

2. A process as in claim **1** wherein cells are produced.

3. A process as in claim **1** wherein virus is produced.

4. A process as in claim **1** wherein the propagator is tumbled during the cell plating cycle in order to evenly distribute the cell slurry on both sides of the plates.

5. A process as in claim **1** wherein the propagator is tumbled during the cell harvesting cycle in order to increase the cell concentration.

6. A process as in claim **1** wherein the propagator is tumbled during the virus seeding cycle in order to evenly and optimally infect the virus suspension.

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