Approved by Senior Author
Signature M. K. Patterson Jr.
Date April 19, 1976

PERFUSION AND MASS CULTURE SYSTEMS

Submitted by

M. K. PATTERSON, JR.

Samuel Roberts Noble Foundation
Ardmore, Oklahoma 73401

INTRODUCTION

Perfusion culture systems are almost as old as the technique of tissue culture. Ross Harrison, while working at The Johns Hopkins University, published (1907) the first paper on the growth of nerve fibers in culture. Associated with Harrison was a young medical student, Montrose Burrows, who subsequently joined Alexis Carrel at The Rockefeller Institute. In 1912 Burrows published a description of a perfusion system in *Anatomical Record.* The contributions of Carrel and Lindbergh to the development of perfusion systems for organ culture are well known. In the late 1940's the marked technical advances in microscopy, the advent of antibiotics, and the perfection of tissue culture methods using microbiological techniques fostered many developments in perfusion systems. Between 1951 and the mid-1960's, some twenty designs were described.

Improvement in design directed toward automation and monitoring the systems has continued. Exclusive of their use as labor-saving devices, the utility of such apparatus lies in their application toward stated goals. The major goal is of course to mimic a tissue's environment in defined terms, or as many authors state, "to achieve as near physiological conditions as possible" principally through avoidance of "feast and famine" during periods of maintenance or growth.

The systems vary in size from capillaries to multiliter vessels and in sophistication from simple gravity flow systems to one designed and carried into space on Sky Lab III. Applications vary from those used in vaccine and hormone production and developing and testing artificial vascular prosthesis to the study of "clearance rate" of drugs mimicking "in vivo" clearance. Systems have been designed to perfuse cells (both monolayer and suspension) and tissues, as well as whole organs.

Mass culture systems are included in this section because it is felt that they lend themselves readily to perfusion systems. The advantages of feeding mass cultures by this method are readily apparent with the potential of re-utilization of spent media, use of monitoring devices, and cell harvesting.

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

PERFUSION CULTURES — HISTORICAL (pre-1940)

Vertebrate — Cells and Tissue

Bakker, A. 1936. The behavior of the crystalline lens of the rabbit during *in vitro* perfusion. Nederland. tijdschr. geneesk 80: 3802-3803.

Bakker, A. 1938. The behaviour of a rabbit lens and iris in a perfused culture. Acta Neerl. and Morphol. Normal Path. 1: 97-114.

Burrows, M.T. 1912. A method of furnishing a continuous supply of new medium to a tissue culture in vitro. Anat. Rec. 6: 141-144.

Burton, A.C. 1939. The properties of the steady state compared to those of equilibrium as shown in characteristic biological behavior. J. Cell. Comp. Physiol. 14: 327-349.

Carrel, A. 1931. Maintaining a constant medium in tissue cultures. C. R. Soc. Biol. (Paris) 106: 7-10.

De Busscher, G. 1930. On some details of technique of cell culture *in vitro* with continuous perfusion. Bull. Histol. Appl. Physiol. Pathol. Tech. Microbiol. 7: 333-336.

NOT TO BE DUPLICATED
IN ANY MANNER
Procedure No. 0|8|9|8|5|

De Haan, J. 1937-38. The perfusion method for tissue culture in its latest form. Acta Neerl. and Morphol. Normal Path. 1: 12-23.

Julius, H.W. 1934. A circulation system for tissue culture (modification of De Haan's apparatus). Bull. Histol. Appliq. Physiol. et Tech. Micr. 11: 32-39.

Lindbergh, C.A. 1939. A culture flask for the circulation of a large quantity of fluid medium. J. Exp. Med. 70: 231-238.

Long, J.A. 1939. A pulsating circulation apparatus for tissue cultures, embryos and small organs. Univ. Calif. (Berkeley) Publ. Zool. 43: 211-216.

Romeis, B. 1942. An improved culture system for explants. Z. Wiss. Mikroskopie 29: 530-534.

Schade, H. 1933. On a physiochemical method, tissue cultures maintained in homologous serum without the usual additions. Arch. Exp. Zellforsch. 14: 631-654.

Suy, R. 1931. System for continuous irrigation. Bull. Histol. Appliq. Physiol. et Tech. Micr. 8: 294-298.

von Bahr, G. 1940. The influence of calcium deficiency on the surviving rabbit lens. Acta Ophthalmol. 18: 170.

PERFUSION CULTURES

Vertebrate — Cells

Barski, G., and R. Robineaux. 1956. A dismountable and sterilizable perfusion chamber for long-term tissue cultures. Ann. Inst. Pasteur 90: 514-517.

Berkley, C. 1966. Potentials for automatic control methods in defined media studies. Ann. N. Y. Acad. Sci. 139: 234-242.

Bernstein, E.F. 1972. Evaluation of mechanical systems used in perfusion. Acta Endocrinol. (Suppl.) (Kbh) 158: 44-73.

Bjorklund, B., V. Bjorklund, and J. Paulsson. 1961. The cytostat, an apparatus for automatically controlled continuous propagation of suspended cells. Proc. Soc. Exp. Biol. Med. 108: 385-390.

Briand, P. 1966. Cultivation of monolayer cell cultures in a continuous perfusion system. Acta Pathol. Microbiol. Scand. 66: 31-40.

Buchsbaum, R., and J.A. Kuntz. 1954. The effects of certain stimulants and depressants on individual fibroblasts in a perfusion chamber. Ann. N. Y. Acad. Sci. 58: 1303-1310.

Castor, L.N. 1970. Control of division by cell contact and serum concentration in perfusion cultures of 3T3 cells. J. Cell Biol. 47: 31a. (Abstr.)

Christiansen, G.S., B.S. Danes, L. Allen, and P.S. Leinfelder. 1953. A culture chamber for the continuous biochemical and morphological study of living cells in tissue culture. Exp. Cell Res. 5: 10-15.

Cross, R.B., and J. Trace. 1972. Inexpensive constant pressure pulsatile perfusion pump. Lab. Pract. 21: 351-352.

Cunningham, A.W.B., and B. Estborn. 1958. Chamber and flow system for quantitative tissue culture. Lab. Invest. 7: 156-162.

Cunningham, A.W.B., and W. Herbst. 1960. A simplified quantitative tissue culture flow system. Lab. Invest. 9: 384-387.

Cunningham, A.W.B., K. Kleutch, and W. Herbst. 1960. Effect of supernatant potassium-level on cardiac activity in quantitative tissue culture. Nature 186: 241-242.

Cunningham, A.W.B., and N.O. Lunell. 1961. Further simplification of a supernatant flow system for tissue culture. Experentia 17: 94-95.

de Laforest, P.G., R. Robineaux, and J. Voisin. 1967. Improvements and details of use of a perfusion chamber for organ culture in vitro. Ann. Inst. Pasteur 113: 449-454.

Dick, D.A.T. 1955. An easily made tissue culture perfusion chamber. J. Microscop. Sci. 96: 363-369.

Dirstine, P.H., D.B. MacCallum, J.H. Anson, and A. Mohamed. 1963. A new microscale continuous flow system for tissue culture. Exp. Cell Res. 30: 426-430.

Eijgenstein, L.J., and F.J.A. Prop. 1970. A simple versatile large-surface tissue culture chamber

Approved by Senior Author
Signature
Date April 19, 1976

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER

Procedure No. 0|8|9|8|5|

Approved by Senior Author
Signature
Date April 19, 1976

for phase contrast photography and microcinematography. Exp. Cell Res. 60: 464-466.

Eisler, W.J., Jr., and R.B. Webb. 1968. Electronically controlled continuous culture device. Appl. Microbiol. 16: 1375-1380.

Estborn, D.B., A.W.B. Cunningham, L. Neill, G. Thomas, H.N. Thomas, P. Cochrane, and S.G. Stephens. 1958. Quantitative tissue culture technic as applied to the study of cardiac potentials. Lab. Invest. 7: 524-532.

Fogh, J., and B. Allen. 1967. Time lapse movie chamber for virus and long-term tissue culture experiments. Proc. Soc. Exp. Biol. Med. 125: 777-778.

Folkman, J., S. Winsey, and T. Moghul. 1967. The culture of human leukemia: a simplified perfusion system for the growth of colonies of human neoplasms. Surgery 62: 110-117.

Freed, J.J. 1963. Cell culture perfusion chamber: adaptation for microscopy of clonal growth. Science 140: 1334-1335.

Graff, S., and K.S. McCarty. 1957. Sustained cell culture. Exp. Cell Res. 13: 348-357.

Graff, S., and K.S. McCarty. 1958. Energy costs of growth in continuous metazoan cell cultures. Cancer Res. 18: 741-746.

Himmelfarb, P., P.S. Thayer, and H.E. Martin. 1969. Spin filter culture: the propagation of mammalian cells in suspension. Science 164: 555-557.

Hu, F.N., S.G. Holmes, C.M. Pomerat, C.S. Livingood, and K.P. McConnel. 1951. A method for studying the effect of P^{32} on living adult human epidermal cells in a perfusion chamber. Texas Rep. Biol. Med. 9: 739-748.

Knazek, R.A., P.M. Gullino, P.O. Kohler, and R.L. Dedrick. 1972. Cell culture on artificial capillaries: an approach to tissue culture *in vitro*. Science 178: 65-66.

Knazek, R.A., P.O. Kohler, and P.M. Gullino. 1974. Hormone production by cells grown *in vitro* on artificial capillaries. Exp. Cell Res. 84: 251-254.

Kruse, P.F., Jr., L.N. Keen, and W.L. Whittle. 1970. Some distinctive characteristics of high density perfusion cultures of diverse cell types. In Vitro 6: 75-88.

Kruse, P.F., Jr., and E. Miedema. 1965. Production and characterization of multiple-layered populations of animal cells. J. Cell Biol. 27: 273-279.

Kruse, P.F., Jr., and E. Miedema. 1965. Glucose uptake related to proliferation of animal cells in vitro. Proc. Soc. Exp. Biol. Med. 119: 1110-1112.

Kruse, P.F., Jr., E. Miedema, and H.A. Carter. 1967. Amino acid utilizations and protein synthesis at various proliferation rates, population densities, and protein contents of perfused animal cell and tissue cultures. Biochemistry 6: 949-955.

Kruse, P.F., Jr., B.C. Myhr, J.E. Johnson, and P.B. White. 1963. Perfusion system for replicate mammalian cell cultures in T-60 flasks. J. Natl. Cancer Inst. 31: 109-123.

Kruse, P.F., Jr., W. Whittle, and E. Miedema. 1969. Mitotic and non-mitotic multiple-layered perfusion cultures. J. Cell Biol. 42: 113-121.

Kruse, P.F., Jr., and M.K. Patterson, Jr. (Eds.). 1973. *Tissue Culture: Methods and Applications.* Academic Press, New York. (A compendium of perfusion systems can be found in Section VI.)

Kruse, P.F., Jr. 1974. Production of multilayered tissue cultures. In: S. Fleischer, and L. Packer (Eds.), *Methods in Enzymology. Biomembranes — Cells, Organelles and Membranous Components.* Academic Press, New York, pp. 568-574.

Kruse, P.F., Jr., 1972. Use of perfusion systems for growth of cell and tissue cultures. In: G.H. Rothblat, and V.J. Cristofalo (Eds.), *Growth, Nutrition, and Metabolism of Cells in Culture.* Volume II. Academic Press, New York, pp. 11-66.

Leinfelder, P.J., and B.S. Danes. 1956. Some problems involved in the culture of cells in a perfusion chamber. Anat. Rec. 124: 492-493. (Abstr.)

Letarte, J., A.E. Lambert, and B. Jeanrenaud. 1974. New system for continuous flow incubation (perfusion). Union Med. Can. 103: 667-672.

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER

Procedure No. 0|8|9|8|5|

McCoy, T.A. 1965. The growth of massive cell cultures in a three-dimensional matrix system. In: C.V. Ramakrishnan (Ed.), *Tissue Culture.* Dr. W. Junk Publishers, The Hague, pp. 108-115.

McCoy, T.A., W. Whittle, and E. Conway. 1962. A glass helix perfusion chamber for massive growth of cells *in vitro.* Proc. Soc. Exp. Biol. Med. 109: 235-237.

McLimans, W.F., F.E. Giardinello, E.V. Davis, C.J. Kucera, and G.W. Bake. 1957. Submerged culture of mammalian cells: the five liter fermentor. J. Bacteriol. 74: 768-774.

McLimans, W.F., D.T. Mount, S. Bogitch, E.J. Crouse, G. Harris, and G.E. Moore. 1966. A controlled environment system for study of mammalian cell physiology. Ann N.Y. Acad. Sci. 139: 190-213.

Miedema, E., and P.F. Kruse, Jr. 1965. Enzyme activities and protein contents of animal cells cultured under perfusion conditions. Biochem. Biophys. Res. Commun. 20: 528-534.

Monod, J. 1950. Theory and applications of continuous culture technique. Ann. Inst. Pasteur 79: 390-410.

Morasca, L., and C. Rainisio. 1966. A method for perfusing tissue cultures with an *in vivo* system. Experientia 22: 337-338.

Morgan, W.D., and C.J. Dawe. 1961. An intermittent perfusion and gas exchange method for continuous cultivation of cells in the Rose chamber. J. Natl. Cancer Inst. 26: 133-149.

Nefedov, V.P., and F.V. Krasovsky. 1965. A method for continuous cultivation of suspended animal cells in a circulating system. Biol. Eksp. Biol. Med. 60: 119-121.

Parker, R.C. 1961. *Methods of Tissue Culture.* 3rd edition. Paul B. Hoeber, Inc., New York.

Paul, J. 1957. A perfusion chamber for cinemicrographic studies. J. Microsc. Sci. 98: 279-280.

Paul, J. 1965. *Cell and Tissue Culture.* 3rd edition. The Williams and Wilkins Co., Baltimore.

Penso, G., and D. Balducci. 1963. *Tissue Cultures in Biological Research.* Elsevier Publishing Co., New York, pp. 47-51.

Peraino, C., S. Bacchetti, and W.J. Eisler. 1970. Automated continuous culture of mammalian cells in suspension. Science 169: 204-205.

Pirt, S.J., and D.S. Callow. 1964. Continuous-flow culture of the ERK and L types of mammalian cells. Exp. Cell Res. 33: 413-421.

Pomerat, C.M. 1951. In: M.B. Visscher (Ed.), *Methods in Medical Research.* Volume 4. The Year Book Publishers, Inc., Chicago, pp. 275-277.

Pouchelet, M, and C. Moncel. 1974. Microculture chamber for long-term microscopic observation involving a continuous perfusion and thermoregulation device. Microsc. Acta 75: 352-360.

Richter, K.M., and N.W. Woodward, Jr. 1955. A versatile type of perfusion chamber for long-term maintenance and direct microscopic observation of tissues in culture. Exp. Cell Res. 9: 585-587.

Rose, G.G. 1954. A separable and multipurpose tissue culture chamber. Texas Rep. Biol. Med. 12: 1074-1083.

Rose, G.G. 1957. Special uses of the multipurpose tissue culture chamber. Texas Rep. Biol. Med. 15: 310-312.

Rose, G.G. 1967. The circumfusion system for multipurpose culture chambers. J. Cell Biol. 32: 89-112.

Rose, G.G., M. Kumegawa, H. Nikai, M. Bracho, and M. Cattoni. 1970. The dual-rotary circumfusion system for Mark II culture chambers. Microvasc. Res. 2: 24-60.

Rose, G.G., C.M. Pomerat, T.O. Schindler, and J.B. Trunnell. 1958. A cellophane strip technique for culturing tissue in multipurpose culture chambers. J. Biophys. Biochem. Cytol. 4: 761-764.

Savageau, M.A., and J.P. Steward. 1967. Contraction rate of rat cardiac cells in culture: responses to prolonged perfusion. Curr. Mod. Biol. 1: 159-164.

Schwartz, B. 1960. A critical analysis of the closed system technique for lens culture. Part I. AMA Arch. Ophthalmol. 63: 593-606.

Approved by Senior Author
Signature [signature] *Date* April 9 1976

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER

Procedure No. 0|8|9|8|5|

Approved by Senior Author
Signature
Date April 19, 1976

Schwartz, B. 1960. The design and performance of a perfusion system for the culture of the lens. Part II. AMA Arch. Ophthalmol. 63: 607-624.

Schwartz, B. 1960. Development of a synthetic medium for rabbit lens culture in a perfusion system. Part III. AMA Arch. Ophthalmol. 63: 625-642.

Schwartz, B. 1960. Initial studies of the use of an open system for the culture of the rabbit lens. Part IV. AMA Arch. Ophthalmol. 63: 643-659.

Schwartz, P.L., R.E.H. Wettenhall, and J. Bornstein. 1968. The growth of newborn rat tibiae in a continuous-flow organ culture system. J. Exp. Zool. 168: 517-530.

Schwobel, W. 1954. A simple stainless steel perfusion system for tissue culture. Exp. Cell Res. 6: 79-86.

Shadomy, S., M.J. Pickett, and S. Froman. 1964. A perfusion culture chamber for phase contrast studies. Am. Rev. Respir. Dis. 89: 581-586.

Shahar, A., et al. 1970. Cultivation of isolated nerve cells in a perfusion chamber and the early effects of nerve growth factor on them. Brain Res. 23: 315-321.

Sharp, J.A. 1959. A modification of the Rose chamber. Exp. Cell Res. 17: 519-521.

Smith, R.E., and F. Kozoman. 1973. Device for automatic rapid harvest of roller culture supernatant fluid. Appl. Microbiol. 25: 1008-1010.

Tarasov, V.N. 1967. Automatic cultivation of cells and viruses. Vopr. Virusol. 12: 93-99.

Thayer, P.S., P. Himmelfarb, and D. Roberts. 1970. Effects of perfusion with amethopterin on L1210 leukemia cells in spin filter culture. Cancer Res. 30: 1709-1714.

Thomas, F., and L. Cramer. 1966. A chamber and perfusion system for general and specialized *in vitro* studies. Texas Rep. Biol. Med. 24: 700-711.

Thomas, F., and L. Cramer. 1966. A chamber and perfusion system for general and specialized *in vitro* studies: biaxially oriented polystyrene: an inert substrate and diffusion membrane for use in tissue culture. Texas Rep. Biol. Med. 24: 712-719.

Toy, B.L., and W.A. Bardawil. 1958. A simple plastic perfusion chamber for continuous maintenance and cinematography of tissue culture. Exp. Cell Res. 14: 97-103.

Ubukata, A. 1966. A perfusion chamber for continuous microscopical observations of living cells in culture. Med. J. Osaka Univ. 17: 137-140.

Willmer, E.N. (Ed.) 1965. *Cells and Tissues in Culture.* Academic Press, New York.

Woodliff, H.J. 1958. A further use of Rose's tissue culture chamber. Texas Rep. Biol. Med. 16: 380-384.

MASS CULTURE TECHNIQUES

Vertebrate — Cells

Barton, M.E. 1971. Effect of pH on the growth cycle of HeLa cells in batch suspension culture without oxygen control. Biotechnol. Bioeng. 13: 471-492.

Bjorklund, B., V. Bjorklund, and J. Paulsson. 1961. The cytostat, an apparatus for automatically controlled continuous propagation of suspended cells. Proc. Soc. Exp. Biol. Med. 108: 385-390.

Bryant, J.C., V.J. Evans, E.L. Schilling, and W.R. Earle. 1961. Effect of chemically defined medium NCTC 109 supplemented with methocel and of silicone coating the flasks on strain 2071 cells in suspension cultures. J. Natl. Cancer Inst. 26: 239-252.

Bryant, J.C. 1966. Mammalian cells in chemically defined media in suspension cultures. Ann. N.Y. Acad. Sci. 130: 143-161.

Cohen, E.P., and H. Eagle. 1961. A simplified chemostat for the growth of mammalian cells: characteristics of cell growth in continuous culture. J. Exp. Med. 113: 467-474.

Cooper, P.D., J.N. Wilson, and A.M. Burt. 1959. The bulk growth of animal cells in continuous suspension culture. J. Gen. Microbiol. 21: 702-720.

Earle, W.R., E.L. Schilling, J.C. Bryant, and V.J. Evans. 1954. The growth of pure strain L cells in fluid-suspension cultures. J. Natl. Cancer Inst. 14: 1157-1171.

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER
Procedure No. 0|8|9|8|5

Earle, W.R., J.C. Bryant, E. L. Schilling, and V.J. Evans. 1956. Growth of cell suspensions in tissue culture. Ann. N.Y. Acad. Sci. 63: 666-682.

Earle, W.R. 1958. Long-term cultivation of animal tissue cells in large cultures. Intersociety Symposium on Cultivation and Use of Animal and Plant Cells. Fed. Proc. 17: 967-974.

Earle, W.R., J.C. Bryant, and E.L. Schilling. 1954. Certain factors limiting the size of the tissue culture and the development of massive cultures. Ann. N.Y. Acad. Sci. 58: 1000-1011.

Earle, W.R. 1962. Recent advances in tissue culture and concluding remarks. In: J.W. Green (Ed.), *New Developments in Tissue Culture.* Rutgers University Press, pp. 1-90.

Fontanges, R., P. Deschaux, and Y. Beaudry. 1971. Apparatus for continuous, large-volume suspended cell cultivation. Biotechnol. Bioeng. 13: 457-470.

Gori, G.B. 1965. Chemostatic concentrated cultures of heteroploid mammalian cell suspensions in dialyzing fermentors. Appl. Microbiol. 13: 93-98.

Gori, G.B. 1965. Continuous cultivation of virus in cell suspensions by use of the lysostat. Appl. Microbiol. 13: 909-917.

Graff, S. 1961. Large-scale growth of animal cells *in vitro*. In: J.W. Green (Ed.), *New Developments in Tissue Culture.* Rutgers University Press, pp. 67-78.

Griffiths, J.B., and S.J. Pirt. 1967. The uptake of amino acids by mouse cells (strain LS) during growth in batch culture and chemostat culture: the influence of cell growth rate. Proc. R. Soc. [Biol.] 168: 421-438.

Jensen, M.D., P.S. Lin, and D.F.H. Wallach. 1973. Comparison of cell growth on various surfaces. In Vitro 8: 415. (Abstr.)

Jensen, M.D., D.F.H. Wallach, and P.S. Lin. 1974. Comparative growth characteristics of vero cells on gas-permeable and conventional supports. Exp. Cell Res. 84: 271-281.

Himmelfarb, P., P.S. Thayer, and H.E. Martin. 1969. Spin filter culture: the propagation of mammalian cells in suspension. Science 164: 555-557.

House, W., M. Shearer, and N.G. Maroudas. 1972. Method for bulk culture of animal cells on plastic film. Exp. Cell Res. 71: 293-296.

Kahn, R.H., W.E. Burkel, and V.P. Perry. 1974. Homeostatic and mass culture technology. J. Natl. Cancer Inst. 53: 1471-1477.

Klein, F., W.I. Jones, B.G. Mahlandt, and R.E. Lincoln. 1971. Growth of pathogenic virus in a large-scale tissue culture system. Appl. Microbiol. 21: 265-271.

Klevecz, R.R. 1975. Temporal structure of S phase analyzed using an automated synchrony system. Biotechnol. Bioeng. 17: 675-694.

Kubitschek, H.E. 1954. Modifications of the chemostat. J. Bacteriol. 67: 254-255.

Lynn, J.D., and R.T. Acton. 1975. Design of a large-scale mammalian cell, suspension culture facility. Biotechnol. Bioeng. 17: 659-673.

McCoy, T.A. 1965. The growth of massive cell cultures in a three-dimensional matrix system. In: C.V. Ramakrishnan (Ed.), *Tissue Culture.* Dr. W. Junk Publishers, The Hague, pp. 108-115.

McCoy, T.A., W. Whittle, and E. Conway. 1962. A glass helix perfusion chamber for massive growth of cells *in vitro*. Proc. Soc. Exp. Biol. Med. 109: 235-237.

McLimans, W.F., F.E. Giardinello, E.V. Davis, C.J. Kucera, and G.W. Rake. 1957. Submerged culture of mammalian cells: the five liter fermentor. J. Bacteriol. 74: 768-774.

McLimans, W.F., D.T. Mount, S. Bogitch, E.J. Crouse, G. Harris, and G.E. Moore. 1966. A controlled environment system for study of mammalian cell physiology. Ann. N.Y. Acad. Sci. 139: 190-213.

McLimans, W.F., C. Horng, B. Kwasniewski, F. Robinson, F. Nussbaum, and S. Gaslani. 1975. Direct "primary" suspension culture of mammalian cells. Proc. Am. Assoc. Cancer Res. 16: 105.

Moser, H., and G. Vecchio. 1967. The production of stable steady-states in mouse ascites mast cell cultures maintained in the chemostat. Experientia 23: 120-123.

Approved by Senior Author
Signature [signature] *Date* April 19, 1976

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER

Procedure No. 0|8|9|8|5|

Approved by Senior Author
Signature
Date April 19, 1976

Novik, A., and L. Szilard. 1950. Description of the chemostat. Science 112: 715-716.

Owens, O.V.H., M.K. Gey, and G.O. Gey. 1961. Growth of cells in agitated fluid medium. Ann. N.Y. Acad. Sci. 58: 1039-1055.

Schleicher, J.B., and R.E. Weiss. 1968. Application of a multiple surface tissue culture propagator for the production of cell monolayers, virus and biochemicals. Biotechnol. Bioeng. 10: 617-624.

Telling, R.C. 1968. The mass propagation of mammalian tissue cells in submerged culture. Lab. Pract. 17: 583-586.

Thayer, P.S., P. Himmelfarb, and D. Roberts. 1970. Effects of perfusion with amethopterin on L1210 leukemia cells in spin filter culture. Cancer Res. 30: 1709-1714.

Van Hemert, P., D.G. Kilburn, and A.L. Van Wezel. 1969. Homogenous cultivation of animal cells for the production of virus and virus products. Biotechnol. Bioeng. 11: 875-885.

Weiss, R.E., and J.B. Schleicher. 1968. A multisurface tissue propagator for the mass-scale growth of cell monolayers. Biotechnol. Bioeng. 10: 601-615.

Wohler, W., H.W. Rüdiger, and E. Passarge. 1972. Large-scale culturing of normal diploid cells on glass beads using a novel type of culture vessel. Exp. Cell Res. 74: 571-573.

Ziegler, D., E. Davis, W. Thomas, and W. McLimans. 1958. The propagation of mammalian cells in a 20 L stainless steel fermentor. Bacteriol. Proc. 48: 72-73.

Additional copies may be obtained from
TISSUE CULTURE ASSOCIATION
12111 Parklawn Drive
Rockville, MD. 20852

NOT TO BE DUPLICATED
IN ANY MANNER

Procedure No. 0|8|9|8|5|