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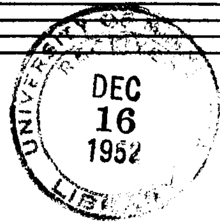

Bulletin of the University of Minnesota Hospitals and Minnesota Medical Foundation

Current Application of Tissue Cultures in Medicine

Volume XXIV | Friday, December 12, 1952 | Number 10

BULLETIN OF THE
UNIVERSITY OF MINNESOTA HOSPITALS
and
MINNESOTA MEDICAL FOUNDATION

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Published weekly during the school year, October to June, inclusive.

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The Bulletin is sent to members of the Minnesota Medical Foundation.
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I. CURRENT APPLICATION OF TISSUE CULTURES IN MEDICINE

William F. Scherer, M.D.

A new diagnostic laboratory method has recently been developed which may, within the next several years, be sufficiently widespread in use to be of great value to practicing physicians. This method, tissue culture, promises to provide an inexpensive, yet accurate means for the diagnosis of virus diseases.

Tissue culture has, for many years, been a technique employed for experimental study by investigators in the medical sciences. Anatomists, physiologists, biochemists, pharmacologists, cancer biologists, pathologists and microbiologists have obtained information by the cultivation of tissues in vitro. Mammalian or avian tissues have been commonly employed, though amphibian and plant tissues have also been studied. For example, Harrison, who initiated work in this field in 1907, utilized amphibian nerve tussue. From 1910 until mid-1930, the center for tissue culture work in America was in Carrel's laboratory at the Rockefeller Institute. The serial passage in vitro from culture to culture, of cells from the heart of a chicken embryo was carried on in that laboratory for a period of over 25 years. It was Carrel and his co-workers who developed many of the basic techniques for the cultivation of cells in vitro.

Today by the application of tissue cultural methods, strains of cells are kept alive in vitro year after year, and are available for experimental use in the medical sciences. This has enabled virologists to employ tissue cultures rather than animals for the study of certain virus diseases. For example, in the Department of Bacteriology and Immunology at the University of Minnesota, the monkey, has been almost completely replaced by cellular cultures for experimentation with poliomyelitis virus.

Methods and Materials Employed for the Cultivation of Animal Cells in vitro

Tissue cultural techniques, originally, were shrouded with mysticism and involved with rituals to avoid contamination of cultures with microorganisms. However, as the use of tissue cultures became more generalized, many of the apparent technical obstacles were replaced by ordinary bacteriological procedures. As a result, tissues are now cultivated chiefly by the employment of equipment found in the bacteriological laboratory.

Three basic methods are employed for the cultivation of cells in vitro: 1) tissue fragments are suspended in a liquid medium; 2) individual cells or small clumps of cells are suspended in liquid; 3) cells are grown on a solid surface.

When tissue fragments or clumps of animal cells are suspended in liquid medium, cells survive for from several days to several weeks. However, very little cellular multiplication occurs, and therefore prolonged maintenance of a cellular population is impossible.

Unlike bacterial cells, animal cells, with one exception, do not proliferate when in suspension. Cells derived by Gey and co-workers from a mouse lymphoblastoma have recently been maintained in vitro in suspensions kept constantly in motion[1].

Aside from this one exception, a generalization can be made that animal cells must be attached to a surface for cellular division to occur. Thus, most methods for the long term cultivation of animal cells in vitro employ a physical substrate. This surface is most commonly either glass or the surface provided by strands of fibrin in clotted plasma. However, cellulose sponge and cellophane have also been utilized.

The shape and size of vessels used for tissue culture have varied greatly. Small specialized flasks, such as the Carrel flask, ordinary test tubes, Erlenmeyer flasks, and hollow ground slides have been employed. The shape of the vessel is relatively unimportant provided it is made from hard glass and is

shaped so that cells are covered by a thin layer of liquid medium.

Two principal constituents must be present in the liquid phase of cultures of animal cells for cells to proliferate. Commonly employed is either serum or a body fluid such as ascitic fluid or aqueous humor. This ingredient provides materials chiefly for the survival of cells although some growth of cells occurs in serum. Either homologous or heterologous serum may be used, though homologous serum is usually superior. The second portion of the liquid medium is a tissue extract that furnishes factors essential for the growth of cells. Commonly aqueous extracts from embryonic tissues are employed. However, extracts from tumor tissues, bone marrow or adult tissues stimulate the growth of certain cells. Since it is unnecessary for the cultivation of animal cells to employ undiluted serum or tissue extract, these materials are commonly diluted so that serum makes up one-third or one-half and tissue extract one-hundredth to one-tenth of the total medium. A solution of inorganic salts, similar in composition to extracellular body fluid is employed as the diluent.

For the growth of animal cells, temperatures of from 30 to 37° C. are essential. The rate of cellular growth increases proportionally to the temperature.

Major Cellular Types

The name tissue culture is, for the most part, a misnomer, for actually, cells, not tissues, proliferate _in vitro_. A more appropriate name is _cellular culture_. Two types of cellular growth occur in cultures: _unorganized growth_, where the arrangement of cells has no resemblance to the original pattern in the tissue, and _organized growth_, where cellular differentiation and histological relationships among cells are maintained.

Unorganized cellular growth _in vitro_ occurs when small pieces of tissue, referred to as explants, are placed in clotted plasma or on a glass surface, and are covered by a serum-extract mixture, for incubation at 37° C. The corona of cells which develops around the explant, results from both cellular proliferation and cellular migration. Within the explant relatively little cellular multiplication occurs, although much of the histological organization of cells is retained.

Three patterns of unorganized cellular growth occur from tissues explanted _in vitro_: 1) a reticular pattern, (a network of spindle-shaped cells or fibroblasts communicating with one another by long branching cellular processes), 2) epithelial growth (polygonal cells forming sheets or membrances, usually one cell in thickness), and 3) an amoeboid pattern (no organization; cells entirely independent of one another). Reticular growth occurs from tissues of mesenchymal origin (i.e. muscle, areolar tissue, sarcomata); epithelial growth from tissues that contain epithelium (_i.e._ skin, liver, intestine, carcinomata); and amoeboid growth from tissues composed of blood or reticulo-endothelial elements (_i.e._ marrow, spleen, lung).

Organized growth and histological differentiation of cells can be made to occur _in vitro_ when attention is directed to the cells within the central piece of tissue rather than to the peripheral corona of cells. Embryologists, in particular, have employed this technique for the study of developing organs and body rudiments.

Most changes in cellular morphology or physiology, that occur when a cell is cultivated _in vitro_, are reversible. Moreover, these alterations do not involve any gain in the number of potential functions of a cell such as occur when cells dedifferentiate. For example, the change, _in vivo_, of seminal vesicular epithelium from columnar to cuboidal epithelium upon castration of an animal is a reversible alteration in cellular morphology, since upon administration of testicular hormone, the epithelium again becomes columnar. If dedifferentiation had occurred, the cells would have reverted to a primitive stage, and become capable of developing not only into seminal vesicular epithelium, but also

into epithelium of the nearby genito-urinary tract organs which had the same embryological derivation. The alterations of cells upon cultivation *in vitro* are thought to be similarly reversible.

A specific function for cells in vitro is often suppressed when the cells grow rapidly, and returns when the cellular growth rate is slowed. For example, embryonic heart muscle cells fail to contract when stimulated to grow rapidly *in vitro*, but contract rhythmically, when grown at a slower rate.

In essence then, cells cultivated from tissues *in vitro* assume a reticular, epithelial or amoeboid pattern of growth. Although cells from different tissues may have identical morphology *in vitro*, they retain their potential to be morphologically and physiologically different.

Applications of Tissue Culture in the Medical Sciences

Tissue Culture in Anatomy - The anatomist recognized early the value of cells cultivated *in vitro* for detailed studies in cytology. For example, tissue cultures made possible continuous cinephotomicrographic observations of living cells in mitosis and of cells under the influence of drugs or irradiation. The thinness of cells in tissue cultures has made them particularly useful for the study of intracellular structures by electron micrography[2].

Transformations of one cell-type to another, such as the conversion of blood monocytes to macrophages, have been studied *in vitro* by the employment of tissue cultures[3]. By the application of histochemical staining methods to monocytes from chicken blood and to macrophages derived *in vitro* from these monocytes, it has been shown that while the parent monocyte is devoid of stainable carbohydrate, lipid or acid phosphatase, the macrophage that develops *in vitro* from the monocyte contains periodic-acid-Schiff positive material and acid phosphatase in the centrosphere or Golgi zone. Thus the macrophage manifests new functional activities as it forms from the monocyte.

The embryologist has used tissue cultural methods to study the development of embryonic rudiments. For example, Fell and Robison[4] cultured the femur from a chicken embryo and showed that normal shape and development of the femur occurred in the absence of stresses from adjacent muscles.

Tissue Culture in Physiology and Physiological Chemistry - When the physiologist applied the methods of tissue culture to the study of muscle physiology, it was soon demonstrated that muscle cells, cultivated *in vitro*, contract even though completely denervated.

The probable explanation for the occurrence of scar tissue at the site of trauma to a muscle, is suggested by observations of muscle and connective tissue cells *in vitro*. Connective tissue fibroblastic cells multiply *in vitro* rapidly after a lag period of only 1 to 2 days. Muscle cells, on the other hand, begin to grow only after 1 to 2 weeks in culture. Thus the delayed lag period for muscle cells probably permits the rapidly growing connective tissue cells to fill in defects at the site of trauma before muscle cells regenerate.

Hormone replacement therapy has been successfully carried out by the implantation of organs maintained *in vitro* into patients with hormone deficiencies. Stone et al in 1934[5], and Kooreman and Gaillard in 1950[6], removed portions of parathyroid and thyroid glands from full term babies, born dead. By tissue cultural methods, parathyroid tissue was preserved for from 30 to 60 days. Upon surgical implantation of the parathyroid tissue into patients with postoperative tetany, parathyroid hormones were secreted by the implanted tissue in quantities sufficient to correct the hormone deficiencies.

Tissue Culture in Pharmacology - Tissue cultures have been used in pharmacology chiefly for two purposes, a) for the evaluation of drug toxicity and b) for determination of the effectiveness of antiviral substances.

Pomerat[7] has listed the advantages of

tissue cultures for drug assays as follows:

1. Tissue cultures are inexpensive when compared with the costs for laboratory animals.

2. Results are obtained in 1 to 3 days.

3. The test systems are genetically homogeneous provided strains of cells, or tissues from one animal are used for a single series of tests.

4. Toxicity of a drug for a variety of cell types derived from different tissues may be determined with equal ease - malignant vs. non-malignant cells, embryonic vs. adult tissues, or a variety of organs. Moreover, human cells may be employed as easily as animal cells.

The use of tissue cultures for the evaluation of antiviral substances is now becoming more wide-spread. For a virus such as poliomyelitis, whose host range does not include the usual laboratory animals, cultures made from susceptible tissues afford an inexpensive means for the evaluation of antiviral substances.

Pomerat[8] has noted that cells _in vitro_ may become "addicted" to morphine. When progressively larger amounts of morphine are added to tissue cultures, tolerance of the cells increases. When morphine is subsequently withdrawn, cells die.

Tissue Culture in Cancer Biology - Malignancy has long been recognized to be a disease that occurs at a cellular level. This fact has led many investigators in cancer biology to study the isolated malignant cell _in vitro_. Early studies showed that malignant cells are independent of host factors for their malignancy, since after cultivation _in vitro_ for long periods of time, they retain the capacity to produce tumors upon inoculation into an animal host. On the other hand, normal cells have become malignant while being cultivated _in vitro_, i.e., the transformation of normal mouse connective tissue cells into sarcomatous cells[9, 10].

Diagnostic classification of certain tumors can be facilitated by cultivation of the tumor tissue _in vitro_. For example, sympathicoblastomas may histologically be difficult to distinguish from lymphosarcoma or Ewing's tumor. Yet, when cultivated _in vitro_, typical neural cells migrate from the tissue to make evident the diagnosis of sympathicoblastoma, usually within 24 to 48 hours[11].

Cultures of tumor tissue have been employed to assess oncolytic effects of drugs and viruses. For example, the oncolytic properties of podophyllin have been studied in tissue cultures[12]. When West Nile virus was added to cultures of human epidermoid carcinoma, destruction of the cells occurred[13]. When West Nile virus was used to infect man with malignant tumors, 4 of 27 patients who became infected with the virus showed transient retardation of tumor growth[14]. Two of these 4 patients had large bowel adenocarcinoma, one a reticulum cell sarcoma, and the fourth patient a thymoma.

Tissue Culture in Microbiology and Pathology - The greatest promise for the practical usefullness of cellular cultures is for the diagnosis of infectious diseases, particularly virus diseases. Yet tissue culture has not been limited in microbiology to the study of viruses. Host-parasite relationships between cells and bacteria, fungi, and protozoa have been studied _in vitro_ in cellular cultures. The cultivation of intracellular bacteria such as _Mycobacterium leprae_ is currently under study in tissue cultures[15] _Histoplasma capsulatum_ has been shown to infect chicken embryonic cells _in vitro_[16]. _Trypanosoma cruzi_, _Leishmania donovani_, toxoplasma and the exoerythrocytic forms of certain plasmodia have been cultivated successfully in tissue cultures[17].

However, the most extensive studies in microbiology that have utilized tissue cultures, concern host cell-virus relationships. The applications of tis-

sue or cellular cultures to virology are listed in Table I. From Table I it can be seen that both theoretical and practical problems in virology have been studied by the use of tissue cultural methods.

TABLE I

Applications of Tissue Culture in Virology

Investigative Problems

1. Tissue and cellular trophism
2. Virus inclusion bodies
3. Metabolism of cells infected with viruses
4. Mode of antibody action upon viruses

Practical Problems

1. Assay of virus
2. Isolation and typing of virus
3. Production of virus
   a) Complement fixation antigens
   b) Vaccines
4. Assay of neutralization antibodies

The identification of various tissues as sites for the multiplication of virus has been accomplished chiefly by the use of tissues cultivated in vitro, since only by such means can tissues be isolated from the host for study. The human viruses that were grown in tissue cultures are listed in Table II[18]. It is apparent from Table II that a variety of human viruses were successfully propagated in vitro in tissue cultures. Since embryonic tissues have the capacity to grow rapidly in vitro, they were employed for the majority of experiments.

An example of the employment of tissue cultures for the study of viral trophism is the identification of human skin, muscle, and intestinal tissues as host tissues, for poliomyelitis virus[19]. These observations would have been difficult to make in man since no lesions occur in those tissues. Yet, by the cultivation of these human tissues in vitro it has been shown that they support the multiplication of poliomyelitis virus, and that the cells are destroyed by the virus.

Likewise, individual cells can be identified in cellular cultures as host cells for viruses. For example, human epithelial cells and human fibroblastic cells have been shown to propagate poliomyelitis virus. The demonstration in vivo that such cells possess the capacity to support the multiplication of poliomyelitis virus would indeed, have been a difficult task.

Tissue cultural methods have been employed for the investigation of virus inclusion bodies since cells cultivated in vitro can readily be examined microscopically as single isolated cells. Recently in our laboratory, the development in vitro of inclusion bodies in rabbit corneal cells has been studied. The experimental results showed that a single corneal cell in vitro can become infected simultaneously with the viruses of pseudorabies and vaccinia and that different morphological forms of intranuclear pseudorabies inclusion bodies occur which are taken to represent developmental stages of the inclusion.

The experimental approach to the metabolism of animal cells by the employment of cells cultivated in vitro is at present in an early developmental stage. Until recently, cultures of single cell-types have not been available for such studies. In the ordinary tissue culture, many types of cells (i.e. - epithelium, fibroblasts, endothelium, etc.) exist. Yet, it is obvious that for metabolic studies, cell types must be studied singly so that the metabolism of other cells does not affect the experimental data. However, recent advances in tissue culture now make it possible to employ strains of epithelial cells and fibroblasts for the study of normal cellular metabolism and of the metabolism of cells when infected with viruses.

TABLE II

Human Viruses Successfully Propagated in Tissue Cultures

Neurotropic Viruses	Host Species	Host Tissues
Anopheles A and B	Chicken embryo	whole embryo
Equine encephalitis	Chicken embryo Rat	whole embryo fibroblasts and sarcoma cells
Herpes simplex	Chicken embryo Rabbit	whole embryo testis, cornea
Ilheus encephalitis	Chicken embryo	whole embryo
Jap. B. encephalitis	Chicken embryo	liver, heart
Lymphocytic choriomeningitis	Chicken embryo Mouse embryo	whole embryo " "
Poliomyelitis	Human embryo Human infant Human adult Monkey - immature Monkey - adult	brain, cord, intestine, skin, muscle foreskin, kidney testis, fibroblasts, epithelium testis, kidney, muscle " " "
Rabies	Chicken embryo Mouse embryo Mouse (to age 14 d.) Rabbit embryo	whole embryo, brain brain " "
St. Louis encephalitis	Chicken embryo Mouse embryo	whole embryo " "
West Nile encephalitis	Chicken embryo	whole embryo
Other Viruses	**Host Species**	**Host Tissues**
Epidemic kerato-conjunctivitis	Mouse embryo	whole embryo
Lymphogranuloma venereum	Chicken embryo Mouse Guinea pig embryo Human thyroid	chorioallantoic membrane, whole embryo brain, spleen, testis brain fibroblasts
Influenza	Chicken embryo	amniotic and allantoic membranes, brain
Mumps	Chicken embryo	amniotic membrane
Psittacosis	Chicken embryo Mouse	whole embryo, muscle, skin, lung spleen
Rift Valley fever	Chicken embryo	whole embryo
Vaccinia	Chicken embryo Chicken Rabbit Guinea pig	whole embryo kidney cornea, testis "
Yellow fever	Chicken embryo Mouse embryo Mouse Guinea pig	whole embryo " " testis, placenta, tumors (sarcoma 37S, carcinoma M63) testis

The failure of antibodies to influence the clinical manifestations of virus infections has been attributed to the inability of antibodies to affect the virus after it invades cells. This phenomenon was demonstrated to occur in tissue cultures. Andrewes[20, 21] showed that antibodies prevented the occurrence of virus III and herpes simplex inclusions in rabbit testicular cells only when added before virus. The cultivation of viruses in tissue cultures has in certain instances, provided inexpensive test objects for viral detection. Recent advances with poliomyelitis virus serve as the best example for this application of tissue cultural methods to virology.

Poliomyelitis virus was first cultivated in 1936 in human embryonic nerve tissue[22] and in 1949, in extraneural human embryonic tissues such as skin, muscles and intestine[19]. Although virus can be detected in cultures of human embryonic tissue by virtue of its cytopathogenic effect, the difficulties in obtaining large supplies of human embryonic tissues prohibited any large scale utilization of these tissue cultures for the diagnosis of poliomyelitis. Experimental studies were therefore carried out with monkey tissues. As a result, testicular tissue was found to propagate virus readily[23, 24]. Moreover, the presence of virus was made apparent by microscopic observation of the cultures, since virus caused marked destruction of cells. Tissue cultures made from these tissues afford a more practical method for the investigation of poliomyelitis in the laboratory for two reasons: a) monkeys are available in large numbers through the facilities of the National Foundation for Infantile Paralysis, Inc., and b) the tissues from one monkey are sufficient for the preparation of from fifty to several hundred cultures. Cultures of monkey testicular tissue therefore, represent relatively inexpensive test materials for the study of poliomyelitis virus.

Four practical problems in poliomyelitis virus research for which tissue cultures are of value, are listed in Table I. Viral assays are performed by the addition of serial dilutions of viral material into test tube cultures of monkey testicular tissue. The highest dilution of virus material that produces cellular destruction is taken as the titration end-point. Isolation of virus is accomplished by the inoculation of cultures with suspensions of feces from patients. Nonspecific degeneration of cells occurs occasionally even though the fecal suspensions are clarified by high speed centrifugation. Therefore, it is essential to show a) that the cytopathogenic agent isolated will pass serially from one culture to another and b) that the presence of poliomyelitis antibody in the cultures will prevent the destruction of cells. In effect, if type specific antibodies are employed, this latter procedure types the virus as Type 1, 2 or 3.

Monkey tissue cultures are capable of producing poliomyelitis virus efficiently, in large quantities, and in a relatively pure state. Virus usually multiplies several thousand-fold in monkey testicular tissue cultures. Studies of the growth curve for Type 2 poliomyelitis virus in cultures of monkey testicular tissue, showed that virus was produced on the average from 5 to 7 days after inoculation of the cultures. The release of virus from the cells was accompanied by destruction of cells. Occasionally two cycles of virus production occurred in a single culture. The first cycle correlated with the degeneration of cells which had grown to surround the explant; the second cycle was thought to represent virus produced by remaining viable cells within the explant. When all cells were destroyed, virus production ceased. Massive production of virus is accomplished by the employment of large numbers of cultures, each containing large quantities of tissue. Virus is obtained relatively free from proteins and lipids, since for the propagation of virus, synthetic solutions or ultrafiltrates of serum are employed. This latter property of tissue culture virus is a distinct advantage over virus derived from infected brain or spinal cord, when virus is to be used for complement fixation antigen[25] or vaccine.

Poliomyelitis antibodies can be detected and quantitated in a patient's serum by inoculation of the serum and a known poliomyelitis virus into tissue cultures. If antibody is present, the cytopathogenic effects of the virus will be prevented. By the employment of serum dilutions, antibody can be quantitated. Prior to the advent of tissue cultures, tests for poliomyelitis neutralization antibodies were performed in mice (for Type 2 virus only) or monkeys. The tissue culture method is not only less expensive than the use of animals but is equally applicable to all three types of virus.

CONCLUSIONS

The cultivation of animal cells *in vitro* is a field which has attracted the interests of investigators in many of the medical sciences. Since studies *in vivo* at the cellular level are often difficult to perform, it has been necessary to isolate cells *in vitro* for many investigative purposes. Tissue culture is the technique for this isolation and maintenance of animal cells *in vitro*. Recent applications of cellular cultivation to virology have shown that cellular cultures will in the near future provide useful diagnostic methods for virus diseases and will furnish viruses for complement fixation tests and probably for vaccines.

REFERENCES

1. Gey, G. O.
   Personal Communication

2. Porter, K. R., Claude, A., and Fullam, E. F.
   A study of tissue culture cells by electron microscopy. Methods and preliminary observations.
   J. Exp. Med., 81:233-246, 1945.

3. Fawcett, D. W., and Weiss, L.
   Cytochemical observations on chicken monocytes, macrophages, epithelioid cells and giant-cells in tissue culture.
   Presented at the annual meeting of the Tissue Culture Association, March, 1952.

4. Fell, H. B., and Robison, R.
   The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated *in vitro*.
   Biochem. J. 23:767, 1929.

5. Stone, H. B., Owings, J. C., and Gey, G. O.
   Transplantation of living grafts of thyroid and parathyroid glands.
   Ann. Surg. 100:613-628, 1934.

6. Kooreman, P. J., and Gaillard, P. J.
   Therapeutic possibilities of grafting cultivated embryonic tissues in man: I. The parathyroid gland in cases of postoperative tetany.
   Acta chir. Neerl. 2:326-335, 1950.

7. Pomerat, C. M.
   Methods in Medical Research, Edited by M. B. Visscher, 4:260-261, 1951, The Year Book Publishers, Inc. Chicago.

8. Pomerat, C. M.
   Personal Communication.

9. Earle, W. R.
   Production of malignancy *in vitro*; mouse fibroblast cultures and changes seen in living cells.
   J. Nat. Cancer Inst. 4:165-212, 1943.

10. Earle, W. R., and Nettleship, A.
    Production of malignancy *in vitro*; results of injections of cultures into mice.
    J. Nat. Cancer Inst. 4:213-227, 1943.

11. Murray, M. R., and Stout, A. P.
    Distinctive characteristics of the sympathicoblastoma cultivated *in vitro*: A method for prompt diagnosis.
    Am. J. Path. 23:429-442, 1942.

12. Ormsbee, R. A., Cornman, I., and Berger, R. E.
    Effect of podophyllin on tumor cells in tissue culture.
    Proc. Soc. Exp. Biol. and Med. 66:586-590, 1947.

13. Toolan, H. W., and Moore, A. E. Oncolytic effect of Egypt virus on human epidermoid carcinoma grown in X-irradiated rats. Proc. Soc. Exp. Biol. and Med. 79:697-702, 1952.

14. Southam, C. M., and Moore, A. E. Clinical studies of viruses as anti-neoplastic agents with particular reference to Egypt 101 virus. Cancer 5:1025-1034, 1952.

15. Hanks, J. H. Personal Communication.

16. Randall, C. C., and McVickar, D. L. Histoplasma capsulatum in tissue culture. Proc. Soc. Exp. Biol. and Med. 77:150-153, 1951.

17. Meyer, H. Methods in Medical Research, Edited by M. B. Visscher, 4:281-283, 1951, The Year Book Publishers, Inc., Chicago.

18. Robbins, F. C., and Enders, J. F. Tissue culture techniques in the study of animal viruses. Am. J. Med. Sc. 220:316-338, 1950.

19. Enders, J. F., Weller, T. H., and Robbins, F. C. Cultivation of the Lansing strain of poliomyelitis virus and cultures of various human embryonic tissues. Science 109:85-87, 1949.

20. Andrewes, C. H. Virus III in tissue cultures. II Further observations on the formation of inclusion bodies. III Experiments bearing on immunity. Brit. J. Exp. Path. 10:273-280, 1929.

21. Andrewes, C. H. Tissue-culture in the study of immunity to herpes. J. Path. and Bact. 33:301-312, 1930.

22. Sabin, A. B., and Olitsky, P. K. Cultivation of poliomyelitis virus _in vitro_ in human embryonic nervous tissue. Proc. Soc. Exp. Biol. and Med. 34:357-359, 1936.

23. Scherer, W. F., Butorac, G., and Syverton, J. T. Cultivation of poliomyelitis virus in monkey testicular tissue. Fed. Proc. 10:417, 1951.

24. Syverton, J. T., Scherer, W. F., and Butorac, G. Propagation of poliomyelitis virus in cultures of monkey and human testicular tissues. Proc. Soc. Exp. Biol. and Med. 77:23-28, 1951.

25. Svedmyr, A., Enders, J. F., and Holloway, Ann. Complement fixation with Brunhilde and Lansing poliomyelitis viruses propagated in tissue culture. Proc. Soc. Exp. Biol. and Med. 79:296-300, 1952.

II. MEDICAL SCHOOL NEWS

Coming Events

December 15	Seminar on History of Medicine; "The Foundations of Twentieth Century Surgery;" Dr. Donald C. Balfour, Professor Emeritus, Department of Surgery, Mayo Foundation and University of Minnesota Medical School; Todd Amphitheater; 7:30 p.m.
December 16	Minnesota Pathological Society Meeting; "Vignettes of Bone Biochemistry;" W. D. Armstrong, M.D.; Owre Amphitheater; 8:00 p.m.
January 8 - 10	Continuation Course in Anesthesiology for General Physicians
January 19 - 24	Continuation Course in Ophthalmology for Specialists
January 26 - 31	Continuation Course in Pediatric Neurology for Pediatricians, Neurologists, and General Physicians

* * *

Continuation Course

A continuation course in Anesthesiology for General Physicians will be presented by the University of Minnesota on January 8 - 10, 1953, at the Center for Continuation Study. Emphasis will be placed throughout the course on anesthesiological techniques available to practicing physicians and surgeons. Regional anesthesia will be dealt with in detail, and the management of shock will also be discussed thoroughly. Special features will include a consideration of the function and operation of a recovery room and a discussion of hypnosis as an anesthetic. Three distinguished visitors will participate as members of the faculty for this course: Dr. Donald E. Hale, Director, Department of Anesthesiology, Cleveland Clinic, Cleveland, Ohio; Dr. O. Sidney Orth, Professor and Director, Department of Anesthesiology, and Professor of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin; and Dr. Daniel C. Moore, Chief of the Anesthesiology Section, Virginia Mason Hospital, Seattle, Washington. The course will be presented under the direction of Dr. Ralph T. Knight, Director, Division of Anesthesiology, and the remainder of the faculty will include members of the staff of the University of Minnesota Medical School.

* * *

Dr. Mellins Accepts Detroit Appointment

Dr. Harry Z. Mellins, Assistant Professor in the Department of Radiology, has accepted an appointment as the Radiologist at Sinai Hospital in Detroit, Michigan, which will open early in 1953. He will also hold an appointment as Clinical Assistant Professor in the Department of Radiology at Wayne University College of Medicine. He will take over his new duties about January 15. Dr. Mellins' leaving means that the University of Minnesota will be losing an outstandingly capable young radiologist. The entire faculty joins in extending to him best wishes for success in his new post.

* * *

Faculty News

On Friday, December 12, Dr. Leo G. Rigler, Professor and Head, Department of Radiology, will address the Laennec Society of Philadelphia. He will discuss "Some Roentgen Observations on the Vascular Pattern of the Lungs."

Dr. Roy G. Holly, Assistant Professor of Obstetrics and Gynecology, recently visited the Departments of Obstetrics and Gynecology at Cornell University, New York Memorial Hospital, and Columbia University College of Physicians and Surgeons in New York City. He also attended the meeting of the Academy of Medicine where he spoke on the subject, "Uterine Bleeding."

III. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
WEEKLY CALENDAR OF EVENTS

Physicians Welcome

December 15 - 20, 1952

Monday, December 15

Medical School and University Hospitals

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; W-612, U. H.

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:30 - Tumor Conference; Doctors Kremen, Moore, and Stenstrom; Todd Amphitheater, U. H.

12:15 - Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - 5:30 Seminar on Fluid and Electrolyte Balance; Gerald T. Evans; Todd Amphitheater, U. H.

4:00 - 5:00 Pediatric Seminar; Thrombocytopenia Purpura; Frances Schaar; Sixth Floor West, U. H.

4:30 - ECG Reading Conference; James C. Dahl, et al; Staff Room, Heart Hospital.

4:30 - Public Health Seminar; 15 Owre Hall.

4:30 - 6:00 Physiology 114A and Cancer Biology 140 -- Research Conference on Cancer, Nutrition, and Endocrinology; Drs. Visscher, Bittner, and King; "Blood Proteins in Mice," G. Miroff; 129 Millard Hall.

5:00 - 6:00 Urology-Roentgenology Conference; C. D. Creevy, O. J. Baggenstoss, and Staff; Eustis Amphitheater.

* 7:30 p.m. Seminar on History of Medicine; "The Foundations of Twentieth Century Surgery;" Dr. Donald C. Balfour, Professor Emeritus, Department of Surgery, Mayo Foundation and University of Minnesota Medical School; Todd Amphitheater, U. H.

Minneapolis General Hospital

9:30 - Pediatric Rounds; Eldon Berglund; Newborn Nursery, Station C.

10:30 - 12:00 Tuberculosis and Contagion Rounds; Thomas Lowry; Station M.

11:00 - Pediatric Rounds; Erling Platou; Station K.

12:30 - Surgery Grand Rounds; Dr. Zierold; Sta. A.

1:00 - X-ray Conference; Classroom, 4th Floor.

2:00 - Pediatric Rounds; Robert A. Ulstrom; Stations I and J.

Monday, December 15 (Cont.)

Ancker Hospital

8:30 - 10:00 Chest Disease Conference

1:00 - 2:00 Medical Grand Rounds.

Veterans Administration Hospital

8:00 - 9:00 Neuroradiology Conference; J. Jorgens, R. C. Gray; 2nd Floor Annex.

9:00 - G.I. Rounds; R. V. Ebert, J. A. Wilson, Norman Shrifter; Bldg. I.

11:30 - X-ray Conference; J. Jorgens; Conference Room, Bldg. I.

2:00 - Psychosomatic Rounds; Bldg. 5.

Tuesday, December 16

Medical School and University Hospitals

9:00 - 9:50 Roentgenology-Pediatric Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:00 - 12:00 Cardiovascular Rounds; Station 30, U. H.

12:30 - 1:20 Pathology Conference; Autopsies; J. R. Dawson and Staff; 102 I. A.

12:30 - 1:30 Physiology 114D -- Current Literature Seminar; 129 Millard Hall.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

4:30 - 5:30 Clinical-Medical-Pathological Conference; Todd Amphitheater, U. H.

4:30 - ECG Reading Conference; James C. Dahl, et al; Staff Room, Heart Hospital.

5:00 - 6:00 X-ray Conference; Presentation of Cases from Ancker Hospital; Drs. Aurelius, Peterson, and Ogden; Eustis Amphitheater, U. H.

Ancker Hospital

8:30 - 9:30 Medical-Roentgenology Conference; Auditorium.

1:00 - 2:30 X-ray - Surgery Conference; Auditorium.

Minneapolis General Hospital

10:00 - Pediatric Rounds; Spencer F. Brown; Stations I and J.

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff; Station F.

12:30 - Grand Rounds; Fractures; Sta. A; Willard White, et al.

12:30 - Neuroroentgenology Conference; O. Lipschultz, J. C. Michael and Staff.

12:30 - EKG Conference; Boyd Thomes and Staff; 302 Harrington Hall.

1:00 - Tumor Clinic; Drs. Eder, Cal, and Lipschultz.

1:00 - Neurology Grand Rounds; J. C. Michael and Staff.

Veterans Administration Hospital

7:30 - Anesthesiology Conference; Conference Room, Bldg. I.

Tuesday, December 16 (Cont.)

Veterans Administration Hospital (Cont.)

8:30 - Infectious Disease Rounds; Dr. Hall.

8:45 - Surgery Journal Club; Conference Room, Bldg. I.

9:00 - Liver Rounds; Drs. Nesbitt and MacDonald.

9:30 - Surgery-Pathology Conference; Conference Room, Bldg. I.

10:30 - Surgery Tumor Conference; L. J. Hay, J. Jorgens; Conference Room, Bldg. I.

1:00 - Chest Surgery Conference; Drs. Kinsella and Tucker; Conference Room, Bldg. I.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III.

3:30 - 4:20 Clinical Pathological Conference; Conference Room, Bldg. I.

Wednesday, December 17

Medical School and University Hospitals

8:00 - 9:00 Roentgenology-Surgical-Pathological Conference; Paul Lober and L. G. Rigler, Todd Amphitheater, U. H.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Surgery Case; O. H. Wangensteen, C. J. Watson and Staff; Todd Amphitheater, U. H.

1:30 - 3:00 Physiology 114B -- Circulatory and Renal System Problems Seminar; Dr. M. B. Visscher, et al; 214 Millard Hall.

4:00 - 5:30 Physiology 114C -- Permeability and Metabolism Seminar; Nathan Lifson; 214 Millard Hall.

4:30 - ECG Reading Conference; James C. Dahl, et al; Staff Room, Heart Hospital.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Eustis Amphitheater, U. H.

8:00 - 10:00 Dermatological-Pathology Conference; Review of Histopathology Section; R. Goltz; Todd Amphitheater, U. H.

Ancker Hospital

8:30 - 9:30 Clinico-Pathological Conference; Auditorium.

2:00 - 4:00 Medical Ward Rounds;

3:30 - 4:30 Journal Club; Surgery Office.

Minneapolis General Hospital

9:30 - Pediatric Rounds; Max Seham; Stations I and J.

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff; Station D.

11:00 - Pediatric Seminar; Arnold Anderson; Classroom, Station I.

11:00 - Pediatric Rounds; Erling S. Platou; Station K.

Wednesday, December 17 (Cont.))

Minneapolis General Hospital (Cont.)

12:00 - Surgery-Physiology Conference; Dr. Zierold, E. B. Brown; Classroom.

12:30 - Pediatric Staff Meeting; Spatial Vector Cardiography; A. Boyd Thomas; Classroom, Sta. I.

1:30 - Visiting Pediatric Staff Case Presentation; Station I, Classroom.

Veterans Administration Hospital

8:30 - 10:00 Orthopedic X-ray Conference; E. T. Evans and Staff; Conference Room, Bldg. I.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker.

2:00 - 4:00 Infectious Disease Rounds; Main Conference Room, Bldg. I.

4:00 - 5:00 Infectious Disease Conference; C. K. Aldrich; Conference Room, Bldg. I.

4:00 - Combined Medical-Surgical Conference; Conference Room, Bldg. I.

7:00 p.m. Lectures in Basic Science of Orthopedics; Conference Room, Bldg. I.

Thursday, December 18

Medical School and University Hospitals

8:00 - 9:00 Vascular Rounds; Davitt Felder and Staff Members from the Departments of Medicine, Surgery, Physical Medicine, and Dermatology; Heart Hospital Amphitheater.

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

1:30 - 4:00 Cardiology X-ray Conference; Heart Hospital Theatre.

4:00 - 5:00 Physiology-Surgery Conference; Todd Amphitheater, U. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

4:30 - ECG Reading Conference; James C. Dahl, et al; Staff Room, Heart Hospital.

5:00 - 6:00 X-ray Seminar; Carcinoma of the Larynx; Frances P. Conklin; Eustis Amphitheater, U. H.

7:30 - 9:30 Pediatric Cardiology Conference and Journal Club; Review of Current Literature 1st hour and Review of Patients 2nd hour; 206 Temporary West Hospital.

Ancker Hospital

4:00 - Medical-Pathological Conference; Auditorium.

Minneapolis General Hospital

9:30 - Neurology Rounds; Heinz Bruhl; Station I.

10:00 - Pediatric Rounds; Spencer F. Brown; Station K.

10:00 - Psychiatry Grand Rounds; J. C. Michael and Staff; Sta. H.

Thursday, December 18 (Cont.)

Minneapolis General Hospital (Cont.)

1:00 - Fracture - X-ray Conference; Dr. Zierold; Classroom.

1:00 - House Staff Conference; Station I.

2:00 - 4:00 Infectious Disease Rounds; C. K. Aldrich; Classroom.

4:00 - 5:00 Infectious Disease Conference; Classroom.

Veterans Administration Hospital

8:00 - Surgery Ward Rounds; Lyle Hay and Staff; Ward 11.

8:00 - Surgery Grand Rounds; Conference Room, Bldg. I.

11:00 - Surgery-Roentgen Conference; J. Jorgens; Conference Room, Bldg. I.

Friday, December 19

Medical School and University Hospitals

8:00 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Medicine Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:45 - 12:50 University of Minnesota Hospitals Staff Meeting; Roentgen Observations in Strangulation Obstruction; Harry Z. Mellins and Leo G. Rigler; Powell Hall Amphitheater.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

3:00 - 4:00 Neuropathological Conference; F. Tichy; Todd Amphitheater, U. H.

4:00 - 5:00 Physiology 124 -- Seminar in Neurophysiology; Ernst Gelhorn; 113 Owre Hall.

4:30 - ECG Reading Conference; James C. Dahl, et al; Staff Room, Heart Hospital.

5:00 - Urology Seminar and X-ray Conference; Eustis Amphitheater, U. H.

Ancker Hospital

1:00 - 3:00 Pathology-Surgery Conference; Auditorium.

Minneapolis General Hospital

9:30 - Pediatric Rounds; Wallace Lueck; Station J.

10:30 - Pediatric Surgery Conference; Oswald Wyatt; Tague Chisholm; Station I. Classroom.

12:00 - Surgery-Pathology Conference; Dr. Zierold, Dr. Coe; Classroom.

1:00 - 3:00 Clinical Medical Conference; Thomas Lowry; Classroom, Station M.

1:15 - X-ray Conference; Oscar Lipschultz; Classroom, Main Bldg.

Friday, December 19 (Cont.)

Minneapolis General Hospital (Cont.)

2:00 - Pediatric Rounds; Robert Ulstrom; Stations I and J.

Veterans Administration Hospital

1:00 - Pathology Slide Conference; E. T. Bell;;Conference Room, Bldg. I.

10:30 - 11:20 Medicine Grand Rounds; Conference Room, Bldg. I.

Saturday, December 20

Medical School and University Hospitals

7:45 - 8:50 Orthopedic X-ray Conference; W. H. Cole and Staff; M-109, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater.

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; Heart Hospital Amphitheater.

9:15 - 10:00 Surgery-Roentgenology Conference; L. G. Rigler, J. Friedman, Owen H. Wangensteen and Staff; Todd Amphitheater, U. H.

10:00 - 11:30 Surgery Conference; Todd Amphitheater, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

Ancker Hospital

8:30 - 9:30 Surgery Conference; Auditorium.

Minneapolis General Hospital

11:00 - 12:00 Medical - X-ray Conference; O. Lipschultz, Thomas Lowry, and Staff; Main Classroom.

Veterans Administration Hospital

8:00 - Proctology Rounds; W. C. Bernstein and Staff; Bldg. III.

8:30 - 11:15 Hematology Rounds; Drs. Hagen, Goldish, and Aufderheide.
11:15 - 12:00 Morphology . . . . Dr. Aufderheide.

* Indicates special meeting. All other meetings occur regularly each week at the same time on the same day. Meeting place may vary from week to week for some conferences.