Advances in CELL CULTURE

VOLUME 1

Advances in CELL CULTURE

Edited by

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VOLUME 1

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PREFACE

Since 1897, when Leo Loeb took the first steps to maintain blood cells, connective, and other tissues outside the body in plasma or serum, there has been a remarkable increase in the volume of published research in the field of cell culture. There are several journals as well as numerous, excellent books devoted primarily or exclusively to this subject. Today cell culture is a scientific discipline which operates far beyond the narrow confines of its original goals.

Surprisingly, no periodical review of this field has appeared as yet, and the aim of this new serial publication, *Advances in Cell Culture,* is to fill this gap. The volumes will have international appeal, and will deal with all aspects of cell culture. "Cell culture," as used in this publication, includes the growth of individual cells or cell populations, the growth of small fragments of explanted tissue, the growth of organs, and the growth of obligate parasites in cell culture systems.

The chapters in this first volume, prepared by recognized authorities in their specialized fields, cover vertebrate, invertebrate, and plant cell culture, as well as the cultivation of obligate parasites. Since they are self-contained, occasional overlap was unavoidable.

Succeeding volumes of *Advances in Cell Culture* will continue to provide critical reviews of important aspects of *in vitro* cultivation and will reflect the increasing understanding of the wide ramifications of *in vitro* techniques. For this task we shall be relying on the continuous cooperation of our colleagues in many countries to review, synthesize, and interpret the advances made in their individual areas of investigation. It is our hope that *Advances in Cell Culture* will reveal from year to year the dedicated quest for the mastery of cell culture and the combined efforts of eminent authorities to evaluate new information so as to benefit all who use *in vitro* techniques as basic and applied research.

I am grateful to the Board of Advisors—Paul J. Chapple, Andreas Dübendorfer, Harry Eagle, Edwin H. Lennette, Toshio Murashige, Keith R. Porter, and James S. Porterfield—who will continue to suggest authors and review topics, thus providing invaluable assistance in the preparation of the volumes in this series. I am also indebted to the staff of Academic Press for their aid in producing this book.

KARL MARAMOROSCH

ROSS GRANVILLE HARRISON
1870–1959

It seems only appropriate to include in this new serial publication *Advances in Cell Culture* a short biographical note devoted to Ross G. Harrison, whose insight and pioneering work led to the foundation of animal cell culture.

Harrison was born in Germantown, Pennsylvania, where he received his early education. Later, when his parents moved to Baltimore, he attended the public schools there, and entered The Johns Hopkins University in 1886 at the age of sixteen. Three years later he received his A.B. degree, having worked primarily in biology, mathematics, chemistry, and physics. By that time, he had mastered not only German but also Latin and Greek, deriving great intellectual pleasures from prodigious reading of old classics, as well as Goethe's writings. At the same time, he became an excellent naturalist, hiking and cycling in Pennsylvania, Maryland, and Virginia. In his Biographical Memoirs, J. S. Nicholas (9) stated that as an undergraduate student Harrison had secured "an awareness of nature in a mature sense, an intellectual curiosity which gave him the broad and yet detached perspective which characterized his thinking during his entire career." Harrison entered the Graduate School at The Johns Hopkins University in 1889 and received his Ph.D. degree in 1894, having also, during this period, studied in Bonn, Germany in 1892 and 1893. In 1895 he returned to Bonn, where he was granted his M.D. degree.

In 1890 Harrison spent his first summer at the U.S. Fish Commission at Woods Hole. There he became associated with H. V. Wilson and E. G. Conklin. In 1894 he went to Bryn Mawr where he taught morphology for one year and became associated closely with T. H. Morgan. He returned to The Johns Hopkins University as an instructor in anatomy and became an associate professor in 1899 in the Medical School. In 1907 he became head of the Department of Biology at Yale. In that year, he published his twentieth paper entitled "Experiments in Transplanting Limbs and Their Bearing on the Problems of the Development of Nerves" (4).

Already in 1902 in the Department of Anatomy at The Johns Hopkins School of Medicine in Baltimore, Harrison successfully used an *in vitro* method to observe on glass the living developing nerve fiber of *Limulus* (2). He was seeking a specific answer to a specific question relating to nerve muscle growth, as succinctly pointed out by Bang (1). He excised a small fragment of medullary tube from a frog, placed it on a cover slip in a drop of freshly removed frog lymph, and permitted it to form a clot. Using a Maximov slide, he then observed in this hang-

ing drop culture the outgrowth of nerve cells. The end of a living, growing nerve was thus seen as the fiber extended during embryonic development from the nerve center out to the periphery. The extensive, beautifully illustrated description of nerve outgrowth was published in 1910 (6).

Before these simple and elegant experiments were designed by Harrison, it was generally assumed that "nerve centers and their peripheral end organs are connected from the beginning of embryonic life by means of protoplasmic bridges and that the development of the nerve fibers consists merely in the differentiation of these preexisting connections under the stimulus of functional activity" (5). Harrison wondered whether a stimulus from the nervous system was necessary in order to start the differentiation of striated muscle fibers. He first performed several experiments in which he took limb buds from abnormal, nerveless larvae, transplanted them to normal tadpoles, and compared the subsequent growth and differentiation of normal and aneurogenic limbs (3). Tadpoles with one normal and one additional nerveless limb were maintained alive for a month, while the transplanted buds gave rise to a pair of legs. Then the embryos and the legs were sectioned and the relationship of the plexus of the nerves of the spinal cords to the nerveless limb was determined (4). Harrison found that all but one of the transplanted limbs contained nerves (4). He then conceived the brilliant idea of performing critical tests *in vitro*. He took fragments of frog tissue from different parts of the body and placed them in a drop of clotted frog's lymph. The tissues survived and grew from the edge of the pieces excised from the medullary tube. In certain instances Harrison observed the outgrowth of nerve cells that grew rapidly, branched out, and ended up with typical growth cones. He concluded that the nerve fiber is the outgrowth of the ganglion cell and that the nerve elements innervating a muscle play no part in its morphogenesis (6).

Many years later, in 1928, with his typical modesty, Harrison stated that the pioneering study was merely an adaptation of the hanging drop culture method used by bacteriologists for many years. "Any originality, therefore, that may be claimed for this work is due to a combination of ideas, rather than the introduction of any particular device" (7).

In 1917 the majority of the Nobel Committee recommended that Harrison be given the Nobel Prize in Medicine and Physiology "for his discovery of the development of the nerve fibers by independent growth from cells outside the organism" (10). However, because of the raging war in Europe, no prize was awarded that year. Sixteen years later, in

1933, Harrison was again nominated for the prize, but the Nobel Committee decided that "in view of the rather limited value of the method and the age of the discovery" an award would not be recommended (10). Instead, the 1933 Nobel Prize was awarded to T. H. Morgan for his brilliant discoveries concerning the function of the chromosome in the transmission of heredity. Needless to say that in subsequent years, with rapidly increasing practical applications of tissue culture (8), the value of Harrison's method became much more apparent. Problems of growth, organ culture, normal and abnormal cell physiology, production of vaccines, applications in microbiology, cell pathology and cell-virus interactions, and more recently the production of monoclonal antibodies and interferons all evolved from Harrison's original experiments.

At the time the United States entered the First World War, Dr. Richard Goldschmidt of the Kaiser Wilhelm Institute worked as a visiting investigator at the Osborn Laboratory at Yale University, where he and Harrison became well acquainted. Goldschmidt became interested in *in vitro* cultivation and, in 1916, started invertebrate tissue culture.

Morgan, Wilson, Conklin, and other prominent scientists and close friends persuaded Harrison to become the editor of the *Journal of Experimental Zoology*. He agreed and edited 104 successive volumes, as well as a special volume in 1955—a total of 105 volumes. He felt that his editorial responsibilities were an asset, keeping him in close contact with developments in the field. At the same time, he was able to maintain his outstanding scientific output, and never permitted administrative or editorial work to dominate his thought at the expense of his research (9).

In 1938, after retiring from Yale, Harrison became Chairman of the National Research Council, a position he held through World War II until 1946. Under his chairmanship the National Research Council became an operating agency, and through his efforts the participation of scientists in government activities became greatly intensified and appreciated. The utilization of scientists in the formulation of problems and in giving advice to government agencies had a lasting effect and impact during the postwar years. Within the National Research Council Harrison "supported, stimulated and guided the divisional chairmen in their efforts to advance human welfare" (9). At the age of 79 he was invited to deliver the Silliman Lectures at Yale. Six years later, in 1955, he fell and seriously injured himself. He never completely recovered and was unable to leave his home until his death in 1959.

I met Harrison for the first time in the summer of 1947 during a

symposium of the Society for Growth and Development. During subsequent Growth Symposia I had the opportunity to speak with him frequently. In 1948, while traveling with him by car from Burlington to New Haven, I heard from him about his early work and thus became greatly interested in cell cultivation. During this trip, at Lake Ticonderoga, I took the accompanying snapshot.

KARL MARAMOROSCH

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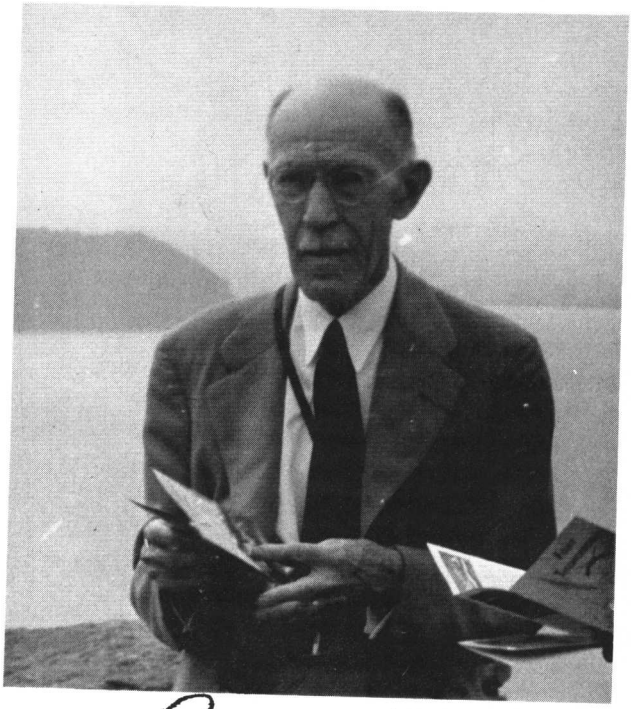

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HYBRIDS AND VIRUSES: REFLECTIONS ON GOLDEN PAST AND LESS CERTAIN FUTURE[1]

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I am still rather puzzled why biologists show such a strong antisexual bias in the consideration of somatic cells. On the other hand, I think I would have been the first to ridicule the fantasy that viruses might carry bits of genetic material from one cell to another in a transductive process, and yet suggestions of this kind seem to be accepted with great gullibility. Projections for future experimentation on somatic cells have invoked transductive phenomena almost to the exclusion of mating. After all, if we combine Stern's discussion with Hauschka's, we will see that every single one of the unit processes needed for the technical handling of mating has been documented in somatic cells. True, they have not been serially documented on a given set of cells under experimental control. But we have reports of the fusion of somatic cells. We know that nuclei of binucleate cells can fuse, if only by coalescence of the spindles at the next mitosis. We know we can have somatic segregation as well as mitotic crossing over. Fifteen years ago we had a much more negative outlook with regard to the possibility of Mendelian analysis with such organisms as bacteria, viruses, and *Penicillium* than we now have for somatic cells.

This was the summary statement given at a symposium on genetic approaches to somatic cell variation held in Gatlinburg, Tennessee, April 2-5, 1958; the author of this comment was Joshua Lederberg (1958). In just a few words, he took exception to the "antisexual bias" shown by biologists in the consideration of mating by somatic animal cells, and predicted the eventual production of somatic cell hybrids. And indeed, only 3 years later, the late Sorieul, technician of the late Boris Ephrussi, mixed cells of two mouse cultures of different origins in Barski's laboratory, and thereby the era of somatic cell hybrids was introduced (Barski *et al.*, 1960).

Although the original hybrids were made by the simple fusion of two

[1]Revised version of talk given at The Waksman Institute of Rutgers University on September 25, 1979 as part of the celebration of the twenty-fifth anniversary of The Waksman Institute.

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kinds of cells of the same species, with the hope that the resulting hybrids would outlive the parental cells, it was much more convenient to devise techniques for endowing parental cells with specific markers, such as deficiencies in certain enzymes, that would prevent their growth in a medium in which the hybrid progeny would grow. It was later found that instead of these imposed markers, cells such as lymphocytes or macrophages, which do not grow in culture, might be used as one of the fusion partners and participate in the formation of hybrids with cells which can be maintained indefinitely in culture. These techniques permitted production of extraordinarily large numbers of cell hybrid cultures. There are no restraints on the species of origin of the parental cells that may be used for the production of somatic cell hybrids. It is as easy to produce hybrids between rat and mouse cells as it is between two mouse cells, and, the number of man × mouse hybrids available is in the hundreds or thousands.

In the 18 years since the "hybrid tool" was invented, it has been responsible for remarkable discoveries in the field of biology. For instance, segregation of human chromosomes in man × mouse hybrid cells has made it convenient for geneticists to assign gene(s) for the expression of a given cell function to a given chromosome of one or another species. The "boom" in this field has been of such magnitude that human chromosomes are "overcrowded" with the genes assigned to them and, at each human genetic workshop, almost as many new genes are assigned to one chromosome as old genes are reassigned to another (Human Gene Mapping 5, Edinburgh Conference, 1979).

The ease with which a stable of somatic hybrid cells was created led to the idea of the use of making hybrids outside the body of the animal "trained" cells for the expression of particular functions. One of the most important needs was to maintain cells trained for the production of antibodies in tissue culture. Since after stimulation with a given antigen in the animal or human body, many B cells respond by proliferation to antigenic stimuli; the resulting antibodies are directed against multiple antigens, and characterization of these antigens, by means of reactivity with such antibodies, encounters certain difficulties. In contrast, if antibody could be produced by the progeny of a single B cell, then only one antigenic determinant of a cell, a virus, a bacterium, or a parasite could be recognized by the antibody and further characterized. Antibodies secreted by progeny of a single B cell (monoclonal antibodies) have been produced by Gerhard *et al.* (1975) against influenza virus, however, it was difficult to maintain for a prolonged period of time production of these antibodies by spleen segments maintained in culture. Hybrid cells (hybridomas) produced as the result of fusion of

antigen-stimulated B cells of permanently growing mouse myeloma cells were able to maintain production of specific monoclonal antibodies indefinitely (Koprowski *et al.*, 1978).

This major breakthrough has led in the last 2 years to the production of thousands of cultures which secrete an antibody geared to deliver new information about cells, viruses, molecules, drugs, chemicals, and so on (Koprowski *et al.*, 1978). The potential of this new biological system is unlimited. Principles laid down by authorities in the fields of pathology, epidemiology, biochemistry, molecular biology, and pharmacology, to name only a few disciplines, will have to be drastically revised in the light of knowledge provided by this new system of identification of biological materials, a system which is much more sensitive than that available until now. Let me quote a few examples.

It has been postulated that recurrent yearly epidemics of human influenza are related to what is called antigenic drifts and shifts in virus strains; according to this hypothesis, any newly arriving virus involved in an epidemic arises because it escaped from the "immunological surveillance" of the host organism. These postulates may or may not be correct; however, the use of monoclonal antibodies produced in tissue culture for the study of variants of influenza virus (Gerhard *et al.*, 1980) is just the beginning of a quantitative and rational approach to the phenomenon.

Rabies virus has been known for over 100 years. Until monoclonal antibodies became available (Wiktor *et al.*, 1977), it was widely accepted that all strains of rabies were the same. However, now we know that marked and significant differences exist among rabies viruses isolated in different parts of the world and from different species. Some humans have died from rabies in spite of having received a complete course of antirabies vaccine ("Human Rabies," 1979; "Viral Diseases," 1976). This may have occurred because the vaccines produced throughout the world are made from one or two strains of virus adapted to the laboratory, and these adapted strains may differ considerably in their antigenic makeup from the virus causing infection (Wiktor and Koprowski, 1980). This difference may account for vaccine failures.

The whole field of study of specific components of human tumors and of immune responses of human subjects to cancers is in a mess because it has been impossible to obtain antibodies with given specificities for cancer antigens. Now panels of monoclonal antibodies are available which are produced by cells grown in tissue culture that recognize antigens specific for a given tumor (Koprowski, 1980). Human melanoma often originates from skin lesions, which are sometimes difficult to classify as malignant lesions ("Human Malignant