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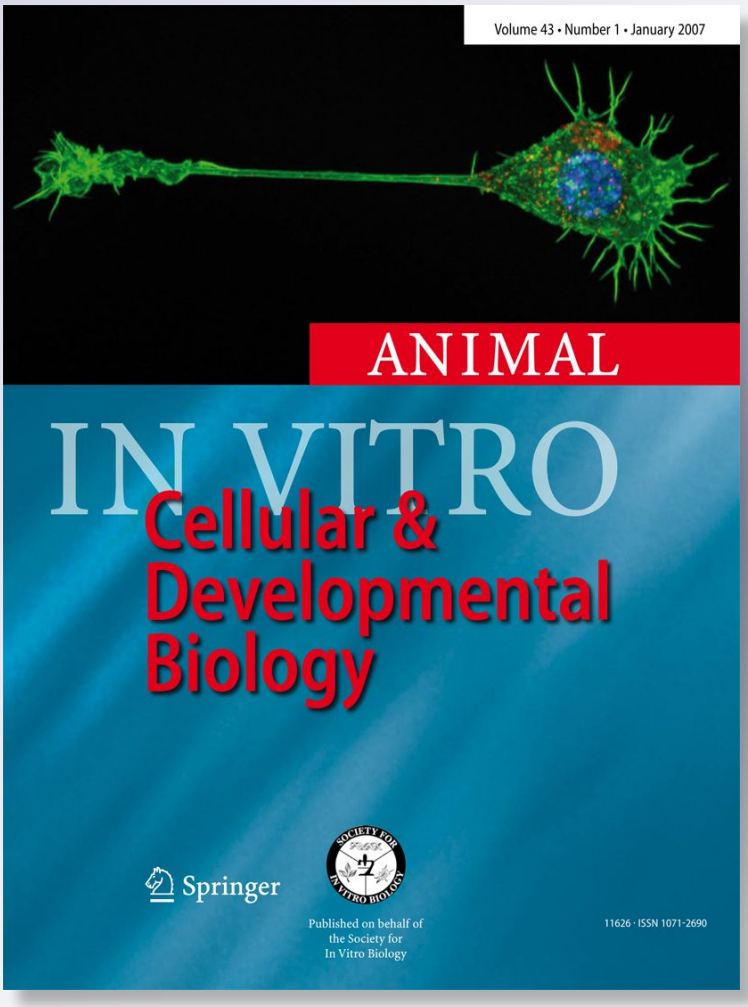

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INVITED REVIEW

A tribute to Richard G. Ham (1932–2011)

Wallace L. McKeehan

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To Teach Is to Touch a Life Forever

Dr. Richard G. Ham (Professor Emeritus, Molecular and Cellular Developmental Biology, University of Colorado, Boulder, CO) passed away on October 28, 2011 at the age of 79. He was a pioneer in the culture of mammalian cells under controlled, defined, and reproducible conditions enabling their use in basic cell level discoveries and practical applications as a production platform and bioassay system.

Dick, as he was known by his colleagues, was born on February 10, 1932 near Morton, WA to dairy farmers Sidney and Mary Laura Ham. He graduated from Queen Anne High School in Seattle, WA and received a bachelor's degree in chemistry from California Institute of Technology in 1953. He began his academic career with a Ph.D. in 1957 in biochemistry from the Department of Chemistry and Biochemistry, University of Texas—Austin. There he did his Ph.D. thesis on mechanisms of hydra regeneration under Professor Robert Eakin. Beginning with Eakin, who discovered biotin-binding avidin and vitamin B12-binding protein and contributed to how folate, biotin, and vitamin B6 works, Dick Ham descends from a long line of chemists that goes back originally to Carl Linnaeus, the father of plant biotechnology and taxonomy (1735). Eakin was a student of nutritional chemist Roger Williams who was a student of Frederick Koch (discoverer of testosterone) who was a student of neurochemist Albert Mathews who was a student of Charles Chandler, the first environmental health chemist and founder of the American Chemical Society. Chandler was a student of Friedrich Wöhler (discoverer of urea, discovered or isolated beryllium, silicon, yttrium, and titanium) who was a student of Jacob Berzelius (originator of chemical formulas, discovered silicon, selenium, thorium, cerium, lithium, and vanadium and coined the word "protein") who was a student of Johann Alfzelius (isolated formic acid and the chemistry of nickel) who was a student of

Addenda by Steven Boyce, Greg Hamilton, Susan Hammond, Stanley McCormick, Donna Peehl, Anne Knedler Shipley, Gary Shipley

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Torberg Bergman, the father of quantitative and carbon dioxide chemistry. Bergman was a student of Linnaeus.

As a student and subsequent research scientist in the Clayton Foundation Biochemical Institute at UT—Austin, Dick's interest in nutritional biochemistry applied to regeneration began while surrounded by pioneers in the field, Roger Williams, Bill Eakin, William Shive, Esmond Snell, and Lester Reed (Ham and Eakin 1958). Attracted by the simplicity of single cell culture for study of nutritional and hormonal responses at the cellular level proposed by Professor Theodore Puck, Dick joined the Puck research group as an Instructor in the Department of Biophysics at the University of Colorado Medical Center in Denver in 1958. He rose to Assistant Professor in 1960. At that time, Puck's laboratory was at the front of application of principles learned from phage and bacteria to animal viruses and animal cells. He actively recruited chemists, biochemists, and phage biologists believing animal cell culture needed their quantitative thinking and awareness of broad issues in biology (Marcus et al. 2006). Max Delbruck's student at Cal Tech and Gunther Stent and Niels Jerne-trained phage geneticist Gordon Sato had joined the Puck lab in 1956. This represented the first overlap of Dick Ham and Gordon Sato that were to be two of the most productive contributors to the definition and development of cell cultures for both practical applications and conceptual discovery to date.

Upon Dick's arrival in Puck's division, Harold Fisher and Gordon Sato (Fisher et al. 1959) had shown that the fetal bovine serum requirement for mass cultures of the most popular human cancer cell line of the day, HeLa, could be replaced by two serum proteins, fetuin and albumin, in a minimal nutrient medium. This was the root of Sato's general concept that the unknowns in mammalian cell culture were largely proteins and hormones that could eventually be identified and purified using the cultured cells as an assay. This gave rise to discovery of novel pleiotropic hormones and tissue growth factors and cytokines that were impossible to identify and characterize via classical endocrinological approaches in vivo. Dick joined the Puck laboratory when Puck's efforts to apply single cell mammalian culture for quantitative assay, genetic selection, and genetic analysis similar to bacteria were in full swing (Marcus and Puck 1958; Tjio and Puck 1958). At that time, mammalian cells with a simple and relatively stable genotype were difficult to culture for long and particularly as single cells for clonal analysis. The Chinese hamster ovary cell line (CHO) was an early experimental target because of its simple and stable near diploid karyotype, short doubling time, high plating efficiency, and mutability resulting in selectable nutrition mutants. Armed with his nutritional background here began with this cell line Dick's signature approach to definition, standardization, and optimization of the cultured cell environment. Single cell clonal culture in the absence of feeder cell layers was a major challenge. Clonal culture reveals the actual requirements of cells under reproducible steady-state conditions where the medium was not modified by cell density and therefore changing moment to moment. However, it also removed the supportive factors and medium "conditioning" provided by neighboring cells at high densities. Using clonal growth of CHO and variant cells as the readout, Dick developed his signature method of systematically optimizing nutritive components of the medium through multiple rounds around a rate-limiting individual component that reduced the serum or less than chemically defined non-nutritive additives in the medium (Ham 1962, 1963). This resulted in the F for fibroblast nutrients series of defined media that culminated with Ham's F12 that facilitated clonal growth of CHO type lines in defined medium in the absence of serum or non-nutritive additives (Ham 1965). Subsequent years in Denver were spent refining the concept and experimenting with applications of single cell analysis in defined medium which contributed to Puck's original objectives of applying bacterial genetics and cloning to mammalian cells.

In 1965, Dick moved to the Department of Developmental Biology, University of Colorado—Boulder which 1968 became the Department of Molecular, Cellular and Developmental Biology (MCDB). Dick participated in creation of the new department as a newly tenured Associate Professor. From cytogenetic studies and lack of reproducibility in single nutrient-deficient media when applied to cells with less exposure time to culture, Dick while still in Denver had concluded that highly optimized nutritive serum-free, protein-free medium was possible because of subtle adaption of the cells accompanied by undetectable genetic changes of near diploid immortal cell lines like CHO and many tumor cell lines to a deficient environment. His first foray into application of the concepts to chondrocyte culture direct from rabbit ear (Ham and Sattler 1968) further illustrated the difficulty ahead in definition of microenvironments for cells from primary tissues and genetically stable cell lines. These lessons gave rise to Dick's fundamental principles that differentiated him from a microbiologist treating mammalian cells as a simple microorganism and distinguished him as a cell biologist focusing on the complexities and specificity of mammalian cell interactions within the microenvironment. Three of his advisory statements that burned in my memory were "adapt the environment for the cells, not the cells to the environment," "every cell and its microenvironment is unique," and "look at the cell and its microenvironment as a dynamic inseparable unit." A preoccupation in the period with application of the new molecular biology and cytogenetic advances to cultured and cloned mammalian cell lines prompted Dick to write one of his comprehensive widely read reviews in this journal, "Nutritional requirements of primary cultures.

A neglected problem of modern biology" (Ham 1974a). This was the guiding pamphlet handed to me when I joined his group in 1974. This was coincident with the increasing attention accompanied with granting agency funding on the utility of standardized karyotypically stable human cell lines derived from embryo or skin for production of biologicals. Serum-free, even chemically defined medium was desirable to eliminate the variability, the potential contamination, and accompanying high cost of fetal bovine serum, a necessary additive at high quantities. Dick put out a national ad for a biochemist willing to tackle the nature of the requirement for serum through reduction of serum to a minimum by nutrient optimization using clonal culture of human diploid cells as assay. This was to be followed by direct identification and replacement of the remaining unknowns by defined non-nutritive components or direct fractionation and biochemical identification of new purified serum factors. This ad, his earlier review above and the conceptual review "Unique requirements for clonal growth" (Ham 1974b), not to mention the prospect of working in Boulder, caught my eye just when recovering from burn out and molecular biological boredom of a postdoctoral extension of my thesis work in translation level protein synthesis. It seemed like an appropriate entry opportunity into multi-factorial cell biology with a biochemical and nutritional approach linking up with the training ground at the Clayton Foundation Biochemical Institute, University of Texas—Austin where both Dick Ham and I begin.

In 1974 with my wife Kerstin McKeehan's technical assistance, we jumped in with full force and enthusiasm to minimize and then replace the dialyzed serum protein requirement for human embryonic diploid fibroblasts either by direct fractionation or replacement with known defined components in serum. We were convinced by this direct approach we would eventually make new discoveries beyond simply empirically developing culture media. Human diploid fibroblasts were the most popular viral substrate and amenable, cytogenetically stable human cell line of the day with a finite lifespan in culture. Ham's F12 was the starting medium and clonal culture the replicate assay to reproducibly indicate the true steady-state quantitative requirements of cells over a 2-wk period without depletion or conditioning by cells. We even from time to time measured directly input and output of key components to prove maintenance of steady-state conditions and stability of the medium over the 2-wk incubation period. Guided by Dick's concepts for systematic nutritional and environmental optimization, we divided non-protein environmental variables up into the physicochemical as gases, pH, organic buffers, osmolarity, and culture substrate/configuration; dietary essential and non-essential amino acids; water soluble and insoluble vitamins and other low molecular weight components including those uniquely required for clonal densities that transport out of cells; and cationic and anionic components of bulk and trace inorganic salts. As novices, we were initially surprised how adjustment of so many individual variables out of each domain significantly lowered the total serum protein requirement, but not Dick who had predicted it all along from his experience and insights over the past few years.

Per the Dick Ham method, the most dramatic or most limiting factor to serum reduction was optimized in order by systematic single variable or component dose responses and then several rounds continued until serum could no longer be lowered. Component concentrations from a dose–response curve were set at the midpoint of the optimum plateau to provide maximum flexibility between deficiency and negative effects of excess (Ham and McKeehan 1978). Particularly problematic were agents with narrow optimum range in water solution as fatty acids and heavy metals which are normally solubilized and buffered in serum by carrier proteins (Bettger et al. 1981). In those days as high as four trays containing about a 6×6 matrix of 25 mm^2 Petri dishes containing 4 to 5 ml of freshly prepared medium minus a single variable was the replicate assay in order to derive enough stainable colonies from a single cell for statistical significance counted first by eye and hand and later densitometry over a 2-wk period. Culture medium minus a single test component was made from concentrated stocks of highly standardized medium components. Medium components were grouped into domains as essential amino acids, non-essential amino acids, water soluble or water insoluble vitamins and chemicals, bulk salts, trace elements, and so on to make background media sans a single component within a test domain. Components known to be of limited stability as reduced cysteine and glutamine were maintained as single independent stocks. Stock media components were strictly dated and turned over, and one of Dick's favorite by the way comments was to inquire about the dates on labile component stocks. Dick ran an antibiotic-free laboratory contending that antibiotic containing medium simply covered up pipetting error and contamination likely to affect quantitative results. Moreover, we did the massive matrix dose–response titration experiments with incubator trays on the open lab bench without hoods. Surprisingly, on average we suffered less than a 10 % random contamination due to pipetting. These efforts were the beginning of the MCDB series of nutrient media named after the department in Boulder (McKeehan and Ham 1976a; Hamilton and Ham 1977; McKeehan et al. 1977; Ham and McKeehan 1979).

Out of these labor-intensive experiments came numerous findings, and the most important of all was the myriad of roles of crude serum proteins in a culture medium both to compensate for deficiencies and to balance out cellular insults and toxicities. One finding was that cells were sensitive to oxygen concentrations even at mile high Boulder and more

sensitive to concentrations at sea level at clonal densities and as serum protein content was lowered (McKeehan et al. 1976). This gave rise to the rumor that the reason sea level groups could not get the Boulder results must have been to the low oxygen in mile high Boulder. Other findings were the substitution of anionic surface coating serum proteins with basic polymer coated plastic substrates (McKeehan and Ham 1976b) and the control of tryptic damage during cell harvesting by use of low temperatures (McKeehan 1977). The latter revealed the important anti-proteolytic and membrane repair roles of crude serum proteins. In 1978, Dick and I reported a new level of quantitative analysis to analysis of nutritive substrates on growth of cells in culture using steady-state Michaelis–Menten kinetic analytical principles to reveal the interactions of medium divalent cations calcium and magnesium and its alteration in tumor cells (McKeehan and Ham 1978).

When Dick moved to Boulder from Denver, he no longer could reproducibly grow CHO cells in serum-free F12 medium. He suspected that this might be due to some unidentified component removed by the evolution of more and more purified bulk nutrient medium components as amino acids and salts. Thus, we embarked on a major series of experiments in collaboration with the analytical chemistry groups in Boulder to analyze the elemental content of ultrapure and quite expensive individual medium components with less expensive sources most commonly used in culture media. The results surprisingly revealed that the use of ultrapure chemicals not only increased the requirement for amount of serum proteins in the medium but in some conditions failed to support the cultures altogether at high levels of dialyzed chelator-treated freeze-dried serum proteins. This motivated the screen for trace metal components in the ultrapure medium in which it was demonstrated that a number of trace metals implicated in animal studies could be shown to stimulate clonal growth and restore efficacy of minimal levels of serum protein in the medium. We realized another of the myriad roles of serum proteins was to provide trace metal deficiencies or to buffer excesses of particularly essential metal cations. The atomic absorption analyses revealed that the least contamination in medium nutrient sources was the anions in contrast to cationic metals as selenate, molybdate, and vanadate (Ham and McKeehan 1979). We demonstrated for the first time that selenium was an essential requirement at the cellular level and again restored ability of CHO cells to grow in serum-free medium (McKeehan et al. 1976; Hamilton and Ham 1977).

During the entire period, Dick and I continuously pursued the replacement of crude serum with bioactive small molecules, culture configurations and substrates, classical hormones, as well as direct purification of serum using our most optimized medium and clonal growth as assay. This went without much success in further decreasing crude serum or increasing specific activity of serum fractions for cells with a limited lifespan in culture or those with differentiated functions (McKeehan et al. 1978). We learned the hard way that serum was an impractical, if not impossible, starting material for direct identification by biochemical purification of defined bioactive proteins. This is due to the fact that it is nature's perfectly balanced physiological fluid comprised of diverse bioactive products from practically every tissue in the body spanning picomolar to millimolar levels with both positive and negative effects on particular cells. Fetal serum was a little simpler presumably because of lack of the full repertoire of factors from the immune system and mature organs. The presence of potent hormone-like regulatory factors at pico- to nanomolar levels that act at picomolar levels in forms from precursors to degradation products influenced by binding protein chaperones and carriers at millimolar levels made it near impossible to concentrate a single active species based on activity. Cell-free serum after clotting not only contains potentially beneficial released platelet-derived factors but also is a balanced mixture of proteases and anti-proteases neutralizing each other to physiologically control destructive effects of clotting in the cascade on serum factors and cells. Once fractionated, their individual unrestrained unbuffered slow release effects are unleashed. In essence, attempts to fractionate intact blood serum created a pathological fluid that subverted the utility of the sensitive clonal culture monitor of bioactivities in the absence of the internal buffering effects ("conditioning") of high density cultures.

In this period, we began to interact with Gordon Sato and colleagues from UC San Diego at meetings who also visited the laboratory in Boulder at an increasingly frequent pace rekindling the relationship between Dick and Gordon that had begun in Denver years earlier. Gordon introduced us to his ideas of utilization of cell cultures to study endocrinology at the cellular level, that a major role of serum reflected physiological hormone action, and that defined cell cultures could be used to discover new hormones that was otherwise impossible using classical endocrinological approaches in vivo. Concurrent reports were emerging that serum and some tissues contained insulin-like factors more potent than insulin on cell growth (Van Wyk et al. 1974) and that blood platelets were a concentrated source of growth factors released into crude serum (Antoniades and Scher 1978). Sato and colleagues were showing that the serum requirement could be reduced or replaced in a variety of differentiated cell lines and some short term primary cultures by mixtures of classical hormones that circulate in serum (Hayashi and Sato 1976; Mather and Sato 1979; Taub and Sato 1979). The medium "conditioned" by cells at sufficient density was shown to be a potent source of diverse growth factors for single cells explaining why clonal growth of single cells was much more difficult than denser culture (Dulak and Temin 1973; Andersson et al. 1977; Burgess et al. 1977). Some of the most potent activities were hormones derived from pituitary or

neural tissue required at levels higher than expected from conventional endocrinology (Gospodarowicz et al. 1974; Nishikawa et al. 1975; Maciag et al. 1979). Separation of the more potent activity from pituitary luteinizing hormone gave rise to the essentially ubiquitous 22-member family of cellular regulators called fibroblast growth factors (FGF) (McKeehan et al. 1998). During this period also first emerged the concept that the action of classical hormones on growth and cell functions might be mediated or supported by more direct acting factors produced by the target cells themselves within tissues (Daughaday et al. 1972; Meier and Hay 1975; Ross 1975; Vaheri et al. 1978).

Influenced by these convergent events, Dick and I adopted the Sato "in vitro endocrinology" approach to identify hormone-like regulatory factors and replacement of the remaining undefined components in highly optimized nutrient media and culture conditions for specific cell types and objectives. Per classical endocrinology, we prepared a diverse library of potential concentrated sources of factors from tissues and cell's "conditioned" medium representing the ablated endocrine organ whose factors were missing in maximally defined clonal cell cultures. Bio-responses in clonal cultures in optimized media containing minimal serum replaced the target tissue examined in vivo in classical endocrine approaches. Out of these early experiments emerged the general efficacy of pituitary or brain extracts as one of the most potent additives for further reduction of serum and useful in support of new specialized cell types that were impossible to establish at normal levels of serum. Although still a challenge because of their extremely low levels, tissue extracts proved a much more realistic starting source for monitoring and purifying bioactive hormone-like polypeptide factors and cytokines which largely became lumped under the term "growth factors."

At this juncture, I departed Dick's research group to take an independent position at the W. Alton Jones Cell Science Center in Lake Placid, NY firmly grounded in both the principles of cell level nutrition and metabolic homeostasis represented by Dick Ham and the cell level regulatory endocrinology and general cell biology represented by Gordon Sato. As he has done for numerous others at different professional levels, Dick provided an important opportunity, environment, and foundation for a topical change in my professional career from hardcore chemistry/biochemistry to cell biology in a patient and nurturing environment. He allowed and promoted complete independence of thought, direction, and self-discovery providing advice from his unprecedented grasp of multi-variate concepts and cellular response networks to the environment whenever one got too far off track. To have been mentored at a "post"-postdoctoral career level by both Dick and Gordon Sato at this point of transition of biology from empirical single variable molecular biology into multi-variate dynamic cell biology was indeed a unique experience and foundation.

After my departure, Dick continued his training and research mission applying the new developments to refinements in defined conditions for human diploid fibroblasts (Bettger et al. 1981; Walthall and Ham 1981), the most widely utilized mouse 3T3 cell line in cell cycle studies (Shipley and Ham 1981; Shipley and Ham 1983) and long-term growth and differentiation of cells direct from specialized tissues with a strong emphasis on human cells. These included human keratinocytes (Peehl and Ham 1980; Tsao et al. 1982; Boyce and Ham 1983) employed in orthologous skin grafts, chondrocytes (Jennings and Ham 1983a, b), preadipocytes (Broad and Ham 1983), mammary epithelial cells (Hammond et al. 1984), and endothelial (Knedler and Ham 1987; Knedler et al. 1989) and muscle cells (Ham et al. 1988, 1990; St. Clair et al. 1992). Some of these contributions are chronicled in more detail by contributors in the Addenda below. Dick continued his intense dedication to undergraduate education and personal advising within the department at Boulder until his retirement to Professor Emeritus in 2001. His largely unheralded service in gladly taking on hard, unpopular, and thankless tasks for the department in Boulder contributed to its long-term growth and excellence over the years.

Although Dick Ham was personally a reserved and unassuming personality with a characteristic deadpan sense of humor, he was a passionate educator at all levels with both a broad and detailed knowledge of his subject. This was independent of whether his subjects were beginning undergraduates, advanced postgraduates, or the scientific literature. He was a course coordinator and author of manuals for Ted Puck's advanced education courses on cell culture in Estes Park in the early 1960s and frequent participant in the advanced courses and symposia at the W. Alton Jones Cell Science Center in Lake Placid, NY, then operated under the Tissue Culture Association (TCA) which is now the Society for In Vitro Biology (SIVB). Dick engaged me while in his research group to step in for him in Lake Placid both in lectures and laboratory instruction which forged the link that ended in my position as a faculty scientist in the institute, involvement in its evolution to independence and establishment of Upstate Biotechnology, Inc. with Gordon Sato, as well as lifelong association with activities of the TCA/SIVB. This included a 14-yr stint as Editor of *In Vitro Cellular and Developmental Biology—Animal*. Dick was the recipient of the Lifetime Achievement Award conferred by the TCA/SIVB in 1991.

Up until his death, Dick continued his love for sharing his broad knowledge with others via the Internet often from his kidney dialysis chair. On one forum that touched on genetics, evolution, and intelligent design, he was known as "Genewatcher," "an articulate, gracious, and very knowledgeable defender of science in general and biology in particular" and "brilliant and special—and a different sort

of man from what usually comes to post on forums" as described by fellow discussants including those that fiercely disagreed with him. He was outspoken on need for quality in science educators: "…..Rapid completion of teacher training is a worthy goal, but not when it excludes CU's outstanding science major programs. The State Legislature urgently needs to revise section 23-1-121 C.R.S. to accommodate programs requiring up to four full years of classes in addition to a semester of student teaching…." (from September 8, 2010 letter to editor of Boulder Daily Camera).

Dick was a private person who separated family and social life from his professional academic work. Privately he was a family man of diverse personal interests in the Boulder, CO foothills where he lived for 47 yr. This included hiking, biking, the welfare of the general Boulder campus environment, and application of his logical scientific thinking to daily life including do-it-yourself home repair. Dick married Mary Linstrom on June 19, 1953 who selflessly supported his scientific and educational endeavors. He is survived by his sons David Hamlin and wife Elizabeth, John Ham and wife Vicki, daughter Carol Tucker, and numerous grandchildren and great grandchildren. A son Paul Ham preceded him in death.

Dick Ham was a man of service whose actions touched many lives that leave him a legacy that he never knew or wished to be given credit. His persistent dedication to the relatively lack luster simple technology of providing support for cells to live out of the body for isolated studies under defined reproducible conditions extends unheralded to thousands now and in the future who put cells in culture media. This is the way he would have wanted it.

Wallace McKeehan did a second postdoctoral with Dick Ham 1974–1978 and is J. S. Dunn Texas A&M Regents & Distinguished Professor in the Center for Cancer & Stem Cell Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center in the Texas Medical Center in Houston, TX. He was Associate Director of the Gordon Sato program at the W. Alton Jones Cell Science Center, a co-founder of Upstate Biotechnology, Inc. and the recipient of the 2012 Lifetime Achievement Award from the Society for In Vitro Biology.

Addenda

Richard G. Ham: a gentle giant of contemporary cell science

Steven T. Boyce

Having come from Ohio to the University of Colorado in 1972 as an undergraduate student in biological sciences, I decided to take a course in the newly formed Department of Molecular, Cellular and Developmental Biology, or "MCDB" as it was called. MCDB was alive with excitement and frequent discoveries compared to the relatively dry taxonomy and ecology of the traditional biology department. After all, it had been less than 20 yr earlier that the structure of DNA had been determined. Some years later, through a circle of friends from Ohio living in Boulder, I met Gary Shipley, who was studying for a Ph.D. with a professor in MCDB, named Dr. Richard Ham. After learning from Gary about the projects for cloning human cells in Dr. Ham's research lab, I applied for a position as a staff researcher there. With no particular enthusiasm, Dr. Ham hired me in 1979. I could not have imagined then that the position with Dr. Ham would irreversibly change my life and those of many people who I was yet to meet.

I was assigned to continue the work of a graduate student, Donna Peehl, who was finishing her Ph.D. on studies of clonal growth of normal human epidermal keratinocytes, which were considered somewhat fastidious to grow at that time. Besides Donna and Gary, there was a remarkably productive postdoc in the lab, named Wally McKeehan, his wife Kerstin, followed later by Mary Tsao, Ben Walthall, Bill Bettger, Ann Knedler, Susan Hammond, and Susan Jennings among others. In this clever company, I learned from Dick Ham the principles of formulation of serum-free and biochemically defined nutrient media (Ham and McKeehan 1979). These were principles taught to him by his postdoctoral mentor, Theodore "Ted" Puck, Ph.D., in the Department of Biophysics at the University of Colorado Medical Center. It was in Denver that Dick formulated *Ham's F12* medium (Ham 1965) which is still used commonly today. Among these bright minds that Dick had brought together, I contributed to formulations of the MCDB series of media for culture of normal human epidermal keratinocytes. To my pleasant surprise, we established a system to isolate, expand, cryopreserve, and clone epidermal keratinocytes in serum-free and biochemically defined media (Boyce and Ham 1983). One day, with some fresh results on the benchtop, Dick came to my bench for discussion. After he explained the biochemical basis for increased cell growth and the elimination of an apparent "requirement" for transferrin, due to availability of ferrous (reduced) rather than ferric (oxidized) iron, he remarked (to paraphrase), "Well done, you must always be prepared for serendipity!" In fall of 1981, I was accepted into the Ph.D. program in MCDB, and Dick agreed to be my thesis advisor. He taught me how formulate hypotheses, design experiments, analyze data, and to write more clearly and concisely. These skills were the engine that would help to propel my career path. The fuel was the knowledge of cellular and developmental biology that he shared with me.

But by then, I was driven with a desire to apply this powerful new technology to medical benefits. In the early 1980s, there were emerging reports from Boston of "cultured

skin," consisting of multi-layered keratinocytes, and "artificial skin," consisting of lyophilized collagen and chondroitin-sulfate biopolymers, being used to treat life-threatening burns. With Dick's patience and willingness to explore, he introduced me to some of his faculty colleagues at the University of Colorado Medical Center where he had studied 20 yr before. There, I began collaborations with several dermatologists, including David Norris, MD, and Richard Clark, MD, and two burn surgeons named John Hansbrough, MD, who directed the burn unit, and John Boswick, MD, a founder of the American Burn Association. During 1983–1984, degradable biopolymer scaffolds were fabricated at the Medical Center in Denver on Mondays, Wednesdays, and Fridays and were populated with keratinocytes in Boulder on Tuesdays, Thursdays, and Saturdays. At the time, I was not aware that Dick Ham was teaching me about the fields of cell therapy, tissue engineering, and regenerative medicine which were yet to be defined. I believe this was an important part of Dick's genius, to support and promote discovery rather than to compete for it. At the community level, it was the same principle as he used in cellular biology, to create 'permissive conditions' for natural processes to operate, rather than imposing human will into systems that were not fully understood. Although he was supportive of biomedical applications from his laboratory, his heart was in basic science research and teaching at which he was skilled and for which he was widely respected. Dick Ham was a scientific purist and consequently made discoveries that were unique because of his singular combination of basic science knowledge, insights, patience, and persistence. With bittersweet reluctance, I left Dick's lab in December 1984 for my first faculty position at the University of California San Diego (UCSD). Earlier that year, based on patents co-authored with Dick, I co-founded Clonetics Corporation which has helped to disseminate the MCDB series of nutrient media from then until now.

In the Department of Surgery at UCSD, and later at the University of Cincinnati and Shriners Hospitals for Children, the principles of cell culture in biochemically defined media became parts of protocols for engineered skin substitutes that have been tested successfully for treatment of life-threatening burn injuries (Hansbrough et al. 1989). These catastrophic injuries bring their victims to the edge of mortality, and some do not return. But resting on the principles from Dick Ham's discoveries and knowledge, it has now been shown that pediatric patients with full-thickness burns involving more than 90 % of their bodies can have their wounds closed (Boyce et al. 2006) and recover a quality of life that allows them to return to their childhoods and their families and to enjoy life again. It has been a privilege, an honor, and a pleasure to be an emissary of discoveries from Dick Ham to advances in clinical care. Today, tomorrow and thereafter, as the frontiers of regenerative medicine continue to advance, I shall always remember and refer the beneficiaries of those advances to a visionary pioneer with a keen mind, a gentle hand, and a kind heart, Richard G. Ham, Ph.D.

Steven Boyce was a Ph.D. student 1981–1984 and co-founder of Clonetics Corporation. He is currently a Professor in the Department of Surgery, University of Cincinnati and a Senior Research Investigator at the Shriners Hospital for Children in Cincinnati, OH.

Dick Ham: a gift to students

Greg Hamilton

I consider myself very lucky to have had the opportunity to do cellular research in Dick Ham's research group and to have taken two of his courses while a student in the MCDB department in Boulder. Dick was shy, gentle, and caring. Those do not seem to be the average and perfect qualities one encounters with most teachers, but I found that the two courses that I took from him, Developmental Mechanisms and Mechanisms of Aging, were two of the most fascinating and informative that I have ever taken. Obviously he did a tremendous amount of research of original papers in those pre-internet days to put together all of the information he served up. Learning from him was exciting, never dull.

Working in his lab was a cherished time. He always had time to give us gentle, thoroughly knowledgeable, guidance. At the time, I did not quite appreciate how cutting edge the work was that was coming out in his laboratory because his personality was not one to inflate or sell his knowledge and vision that is so common a practice today. He was so cerebral and devoted to advancing the science of cellular nutrition. Dick Ham was a wonderful gift to all.

Greg Hamilton worked in Ham's group as an undergraduate student in 1973–1974 and after graduation in 1974–1975. In 1975, with Dr. Ham's help and guidance, he entered graduate school at UT—Austin and the Clayton Foundation Biochemical Institute. Today, he is a businessman in Farmington, NM.

Unheralded contributions to specialized cell cultures

Susan Hammond

At the time I joined Dick Ham's Lab in the late 1970s, cell culture was dramatically different than it is today. At that time, virtually all cell culture was done using immortalized cell lines generated from tumor tissue. The standard media used, DME and the RPMI series, were only functional when supplemented with 10–15 % whole fetal bovine serum (FBS) or horse serum. In addition, cultures were typically

in dense monolayers, subcultured or split with trypsin at the most at a 1:10 ratio. The cells were not normal phenotypically; most of them had the general characteristics of fibroblasts. Even when researchers attempted to start with "pure" populations of epithelia, smooth muscle, or endothelial cells, the cultures would soon be overgrown by the subpopulation, even if very low in number, of fibroblasts. Unknown to researchers at the time, the very use of FBS was part of the problem in that factors such as FGF and TGF-beta that were stimulators of fibroblast growth were high in concentration in serum. In addition, the basal media formulas used had not been optimized for growth of normal, non-stromal cell types such as epithelia and in fact because these media were used with high levels of serum, any inadequacies in their formulas were masked by the serum. Not until the mid-1980s did researchers begin to notice that not only did serum factors promote fibroblasts but that in fact the especially high levels of TGF-beta in serum actually pushed other cell types into early senescence.

As I look back from the vantage point of today's cell culture science where more and more laboratories are using normal human primary cells from a variety of tissues, epithelia, endothelial, osteoblasts, and neural cells of many different types, I realize how important the contributions of Dick Ham were. Such incredible progress has come about by the efforts of this man who was not often credited enough as to what he created. His perseverance in using his simple but elegant single cell clonal outgrowth model allowed us to see the unique requirements of different cell types and change the course of cell biology away from using tumor cells, cell lines, and fibroblasts to the increasing variety of normal human cell types we can now study as well as the teasing out of the differentiation and lineages in stem cell populations.

Dick first began this process by painstakingly optimizing each component in the basal media, asking cells to grow in sparse conditions with less and less serum so that he could see beyond the self-conditioning of dense monolayer cultures and the masking effects of serum. His initial formulation, Ham's F12, became the ground zero from which he went on to work directly with human keratinocytes, then human mammary cells, and then endothelial cells. Each cell type showed its own unique requirements for optimal growth only by the pains-taking method of clonal growth as single cell populations, ideally as 100 cells inoculated into a 60-mm dish. As the process continued, serum was lessened or eliminated and known growth factors such as insulin and EGF were brought in as supplements. In addition, new, as yet unknown factors were sought from tissue extracts such those of the thymus and pituitary glands. As another means to more fully provide all necessary components needed by a cell, Dr. Ham had his team look at the trace elements such as selenium and zinc.

Titrations were made of each component individually over several log levels. As improvements in plating efficiency were seen, additive modifications were made in the basal formulation. By optimizing the previously known basal components as well as determining new factors necessary for growth such as the trace elements, growth factors, hormones, and the pituitary extract, the need for any serum was eliminated until the MCDB 153, MCDB 170, and MCDB 200 and 300 media were able to grow each of their designated cell types serum-free at clonal densities. Today these basal serum-free formulations are used with and without minor modifications by most researchers using primary normal human tissues. They are the basis of most of the commercial media manufacturers' serum-free formulations, though because they were not patented, many manufacturers do not acknowledge the original source.

Lastly, I want to acknowledge Dr. Ham's foresight in providing an open and creative environment in his lab where postdocs, graduate students, undergrads, and staff technicians worked on different cell types and aspects of the derivation of serum-free media. It was in an interactive and mutually supportive way that ultimately was successful in deriving these media and fostered many within the lab to go on in related careers that have continued to expand the knowledge of new techniques and the availability of media and reagents necessary for the advances we are seeing today. Thank you Dick!

Susan Hammond was a Senior Research Assistant and Lab Manager of Dick Ham's Lab, 1981–1987 and primary researcher on the Human Mammary Cell Project that developed the serum-free medium, MCDB 170. In 1987, she founded Hammond Cell Tech based in Northern California and is currently President. Hammond Cell Tech is a manufacturer of supplements for serum-free cell culture.

Richard Ham: the finest qualities of a teacher

Stanley McCormick

In January 1972, Greg Hamilton and I arrived at the University of Colorado as sophomore transfer students. For two 19-yr-old boys from Durango, the town of Boulder held many distractions (not to mention Vietnam War demonstrations and campus streak-ins), but we were nevertheless very keen to begin our studies in the new Department of Molecular, Cellular, and Developmental Biology (MCDB). At the time, casual dress, including beads and sandals, had become accepted attire for professors in the lecture hall, so we noticed Dr. Ham right away. He would come into class with his characteristic bounding gait, arms full of papers and lecture materials, dressed in slacks and dress shirt and tie. It was impossible not to be charmed by this enthusiastic man

who seemed unfazed by all the turmoil on campus. Greg and I took two classes from Dr Ham: "Developmental Mechanisms" and "Mechanisms of Aging." What remains most vivid in my memory after some 40 yr is the excitement his lectures would inspire in the students. After one mesmerizing lecture in Mechanisms of Aging, I remember thinking that the Hayflick Phenomenon must be one of the great mysteries of the cosmos. It was Dr. Ham's unabashed affection for science that endeared him to Greg and I, as it did to hundreds of students that followed.

Emboldened by his inviting manner, I approached Dr. Ham in the fall of 1972 about working in his lab. He said yes, and that was it—no application, no resume, no interview. At that time, there was no other lab in the entire MCDB organization where a kid like myself with no research experience could walk in, set up a space, and dig right in. At night Dr. Ham would often work late in his office, but if I interrupted him with a question about my cultures, he'd eagerly put down his paperwork, come into the lab, and sit at the inverted microscope and share something of his vast knowledge of cell culture. One of the projects he gave me was to prepare metaphase spreads on glass slides of cells after multiple passages in culture. He had specifically asked that I call him to help with the final steps in preparing the slides. But on the last night of the project, I decided to impress him with my earnestness by finishing the work myself. When he came into my room and saw the finished slides, he seemed crestfallen, but luckily, my preparation had failed, and we worked past midnight together, repeating the slides until they worked.

The following spring, Greg joined me in Dr. Ham's lab, and by the mid-1970s, the lab was flourishing with undergraduate and graduate students, as well as a postdoc, Wally McKeehan, and his wife, Kerstin. There was a great sense of camaraderie among Dr. Ham's lab group that reflected the feeling that we were all pulling toward the same goal: the holy grail of growing differentiated cells in defined media. We believed that the power of Dr. Ham's clonal growth method was sufficient to reveal important subtleties in the biology of living cells. It still seems amazing that 35 yr ago, Dr. Ham's clonal growth assay achieved a level of sensitivity sufficient to detect nanomolar concentrations of selenium (McKeehan et al. 1976).

During the 1980s, I was a resident in anatomic and clinical pathology at Yale. Several labs where I trained were studying malignant and normal cells in culture, and some were starting to use immunophenotyping of live cells as a way to classify lymphomas and leukemias. Many doors opened for me as a result of my cell culture training with Dr. Ham. Later, as a practicing hematopathologist in St. Paul, one of my first tasks was to develop a system for transporting clinical specimens (blood, bone marrow, tissue biopsies, needle aspirations) from outlying hospitals and clinics to a central molecular diagnostic laboratory. Of course, I thought of Ham's F12. I tried it on some test specimens and it worked great. We sent aliquots of F12 all over the region to surgeons, pathologists, and clinics. Soon the term "F12" became a buzzword among local clinicians, many of whom believed it must be essential if they were going to obtain an accurate diagnosis on their specimens. I felt good having this connection between my work in pathology and my days in Dr. Ham's lab, and I am sure he would be pleased to know we used the F12 serum-free! Throughout my career, knowing how to culture cells has allowed me to practice at a level unavailable to most hospital-based hematopathologists. In the molecular diagnostics lab where I work, we have used cell lines to develop novel flow cytometry assays and have created quality control material for various genetic and molecular tests using formalin-fixed, paraffin-embedded cell pellets.

Now it remains for the world of basic science to grapple with the legacy of Dr. Ham's work. But because of the way he lived his life, his passing will be felt as a personal loss for many students and colleagues whose lives he touched. For me it marks the end of a 40-yr span in my life during which it was often a comfort to reflect on this gentle, self-effacing man and know that he was back there in Boulder, like an anchor to my college days, doing his brilliant science, mentoring students, and embodying—almost effortlessly—the finest qualities of what it means to be a teacher.

Stanley McCormick worked as an undergraduate student in Dr. Ham's group in 1972 and 1973 and as a medical student in the fall of 1976. He is a pathologist specializing in hematologic and molecular pathology with 28 yr of practice experience at United Hospital, Saint Paul, MN.

Dick Ham—you know, of Ham's F12

Donna Peehl

Throughout my career, in answer to the query, "With whom did you do your PhD?," I have always responded, "Dick Ham—you know, of Ham's F12," and this has almost unfailingly sparked immediate recognition. Dick Ham did the scientific community a great service by his systematic approach to optimizing media for cell culture, especially of human epithelial cells, from which the majority of cancers arise. At the time that I did my graduate research with Dick Ham in the 1970s, I recall that only a few types of cells such as HeLa, CHO, and 3T3 were routinely cultured. The work of the Ham lab laid the foundation for the ability now to culture a plethora of diverse types of cells. It is fortunate that the funding climate was different in the heyday of the Ham lab because it is very unlikely that such research would be reviewed favorably today. Optimizing medium empirically,

step by tedious step, is not glamorous and would today probably not be considered innovative or possibly even significant. Yet, despite the many contributions of the Ham lab, there is still much to do. I have devoted my career to culturing human prostatic epithelial cells, and to this day, no one including me has been able to devise a culture system that robustly and reproducibly induces differentiation of prostate cells. Furthermore, whether malignant cells from primary adenocarcinomas of the prostate, the second leading cause of death from cancer in men in the USA, can be maintained for any length of time in culture is also questionable. So while there is still room for the likes of Dick Ham in science, as is often said, "We shall not see his like again." I recall Dick as a very kind and generous soul but extremely shy—he was a big bear of a man with a big heart who sort of pressed up against the wall as he walked by giving a shy smile. My days in Dick's lab in Boulder were among the best in my life, and I long for the scientific climate that made endeavors such as Dick's possible.

Donna Peehl was a graduate student with Dick Ham [1975–1979] and is Professor of Urology (Research), Department of Urology, Stanford University School of Medicine, Stanford, CA.

Richard Ham: disseminator of practical ideas

Ann Knedler Shipley

Dick's vision and his depth of knowledge of cell nutrition imparted on me an education and a technology that I have used daily for the last 32 yr. Further, he had a close-knit group of people in his lab, many of whom have overlapping influences in my current life and work. During the 4 yr that I spent in Dick's lab, I had the great pleasure to know and to work with Ben Walthall, Susan Hammond, Donna Peehl, Susan Jennings, Judy St. Claire, Tom Broad, Brigitte Van der Hagen, Gretchen Stein, and most importantly, Gary Shipley who I married and with whom I started Cascade Biologics.

I owe a huge debt of gratitude to Dick. His influence on my life career track has been full circle and comprehensive. Dick hired me in my last year of undergraduate school, taught me about tissue culture medium and the need for cell-type-dependent optimal formulation. At the same time, he introduced me to Steve Boyce, a graduate student at the time, who taught me how to isolate and culture keratinocytes and who, after founding Clonetics Corporation, recommended me for employment there. Clonetics was built on everything that I knew from Dick's lab so the fit was perfect. After I moved on, Gary and I started Cascade Biologics and enjoyed 15 yr of profitable business.

The commercialization of the technology born in Dick's lab has allowed researchers worldwide to have access to normal cells and optimized culture systems that were unavailable without fairly advanced technical knowledge. Although I feel that Dick's legacy was more altruistic than commercialization, this put his revolutionary ideas into practice in the scientific and medical research community.

Ann Knedler Shipley was a researcher in the group [1981–1984] and is a scientist in Eugene PSCS Operations of Life Technologies, Eugene, OR.

Richard G. Ham: scientist, teacher, citizen

Gary D. Shipley

The first time I met Dick Ham was a chance encounter in his office at the University of Colorado in the late summer/fall of 1974. I had come seeking employment, but our conversation quickly turned to bicycles as we discussed his efforts to support more bike paths in Boulder and my failed attempt to ride across the plains from Ohio to Colorado. He was dressed in his typical work clothes (navy blue cotton pants, white short-sleeved shirt with pocket protector and heavy black safety-toe shoes) as he peered at me through what seemed impossibly thick glasses. Although his office had a nice view of the campus below, all that was visible over the tops of the stacks and stacks of scientific reprints were the foothills to the north of town. Little did I know that that meeting would change the entire course of my adult life.

After a subsequent interview in which we actually talked about science, he offered me my first real job as a research assistant in his laboratory, and over the next 8 yr, I had the good fortune of working in Dick's laboratory. It was a busy place in those days. Dick had received a number of grants to study the nutritional requirements of mammalian cells in culture, and my job was to learn how to culture mouse embryo fibroblasts in a reduced serum or serum-free environment. Dick taught me how to dissect and digest mouse tissue, liberating the cells that we would study using clonal growth assays. He taught me how to plan experiments, analyze data, and think critically about the questions that we were asking. It turned out that those stacks of reprints in his office were actually carefully arranged so that he could retrieve appropriate references for his students to read after they had asked seemingly simple questions. He was always patient, never condescending. He reveled in helping with even mundane laboratory tasks like tuning up the old phase microscope or the spectrophotometer that was as large as an old Detroit V8 engine. At the same time, he was working on his other passion, developmental biology, and published *Mechanisms of Development* with Marilynn Veomett in

1980. His breadth of scientific as well as practical knowledge was truly stunning.

Later in life I had the pleasure of traveling with Dick in Japan and organizing a conference on epithelial cell biology which he co-chaired with Howard Green. Although his laboratory was winding down in those days, he never lost his passion for understanding how cells work and how they interact with other cells to form tissues, organs, and organisms. He never missed a teaching opportunity.

Elsewhere in this article I am sure that my former colleagues will chronicle how Dick's achievements changed the course of science, spawned commercial successes, and led to life saving clinical techniques. I wanted to give the reader a glimpse of the man I knew: a humble, caring, teacher, scientist, and concerned citizen. A combination of qualities that is difficult to come by in the current age.

Gary Shipley was a research assistant (1974–1978) and a graduate student (1978–1982) in Dr. Ham's lab. After postdoctoral work in experimental pathology at the Mayo Clinic/Foundation, he was an Associate Professor of Cell Biology at the Oregon Health Sciences University and co-founded Cascade Biologics Inc. Gary is currently retired and living in Eugene, OR.

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