

A TRIBUTE TO DR. THEODORE T. PUCK

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Dr. Theodore T. Puck, a pioneer in mammalian cell culture, somatic cell genetics, and the study of human genetic diseases, passed away in 2005. In tribute to Dr. Puck, *In Vitro Cellular and Developmental Biology—Animal* presents invited remembrances from four colleagues whose associations with Dr. Puck spanned 51 years.¹ (Ed.)



THE GENESIS OF “FEEDER CELLS”: CONCEPT AND PRACTICE

Philip I. Marcus (Graduate student, 1954–1957)

Late in the summer of 1954 I left the University of Chicago, lured by Theodore T. Puck to become his second graduate student in the Department of Biophysics at the University of Colorado Medical Center in Denver. Coincidentally, my first postbaccalaureate job at the University of Chicago was in a laboratory where Ted Puck had led a war-time project on the bactericidal action of propylene glycol vapor on microorganisms suspended in air (Puck et al., 1943). While at the University of Chicago I had gained invaluable experience working as a research assistant for Aaron Novick and Leo Szilard in the Institute for Radiobiology, and then for Paul Talalay in the Ben May Cancer Laboratory. I was privileged to see science practiced in its most noble form.

My invitation to join Ted Puck came during a recruitment lunch when he visited Chicago in early 1954. I was fascinated by the challenge he posed. He wanted to be able to grow single mammalian cells into colonies (clones) with the same high plating efficiency achieved with bacteria by microbiologists. The goal was to create conditions in which 100% of the single cells when plated would form colonies. This high cloning efficiency would allow the quantitative approach enjoyed by microbiologists to be applied to mammalian somatic cells, and make it possible to quantify the number of cells in a population capable of reproduction. This single-cell plating, or clonogenic, assay could then be applied to genetic and metabolic studies involving the action of radiation, viruses, drugs, mutagenic agents, and nutritional and other environmental stresses on animal cells. This was a heady temptation for a recently minted (1953) M.S. student in microbiology. I couldn't resist.

The lifetime achievements of Ted Puck attest to the high degree of success realized in attaining these lofty goals. But, in 1954 when I joined Puck, a major hurdle stood in the way. Reading up on the subject, I noted that Albert Fischer, an early authority on cells in tissue culture, had theorized that it might not be possible to grow cells from the body in isolation from each other because they had evolved to grow under conditions where each cell was surrounded by neighboring cells—a condition not met in the dispersal of cells into single members as required to initiate colony or clone forma-

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tion. He astutely noted: "At present we do not know the minimum of cells required for reproduction" (Fischer, 1946). Although the growth of mammalian cells in mass culture was possible, the lack of growth of single cells into colonies appeared to support Fischer's contention. However, this barrier was overcome in 1948 when Sanford, Earle, and Likely were successful in establishing clones from a mouse cell line, NCTC 929 (Sanford et al., 1948).

Earle and his colleagues had hypothesized that single cells did not grow in isolation because of the loss of a diffusible substance needed in critical concentrations to initiate cell growth. Their test of this hypothesis consisted of confining single mouse cells of the L-strain to glass capillary tubes containing "conditioned" medium from mass cultures of growing cells. The capillary tubes, each confirmed microscopically to contain a single cell, were immersed in a petri dish containing conditioned medium. About four single cells out of every 100 succeeded in replicating to the point where growing cells emerged from the capillary tube and were established as a colony or clone. These pioneering studies established the important principle that single mammalian cells were capable of reproduction, and that clones could be established into lines of cells. However, the low plating efficiency resulting from this procedure and its complex methodology did not allow the design of experiments that could answer the myriad questions still eluding cell biologists (Harris, 1995, p. 47).

When I arrived at the basement laboratory assigned to me in the Department of Biophysics, Roshen Christenson, a postdoctoral fellow, was pursuing Earle's approach using conditioned medium in capillary tubes. My initial assignment was to determine the minimum number of cells required to establish continuous growth in a small volume of medium. I substituted microdrops of conditioned medium for the capillary tubes, thereby technically simplifying the process. Ten to thirty microdrops which contained different numbers of HeLa cells, a line derived from a human cervical carcinoma (Gey et al., 1954), were deposited into glass petri dishes along with pledgets of cotton containing water to maintain a high humidity within the covered dish. At that time, medium contained 10% horse plus 20% human serum. Incubation was at 36.5° C in a mixture of 3–5% CO₂ and 95% air saturated with water. Microdrops that contained ≥ 10 –100 cells/drop (≈ 2 –10 μl medium/drop) invariably developed confluent monolayers that could be propagated indefinitely, whereas microdrops with only 1–5 cells initiated growth about 18% of the time—somewhat better than the efficiency achieved with capillaries. However, we did not achieve the high plating efficiency required to study agents that affected the reproduction of mammalian cells, and the methodology was still cumbersome. Clearly, another approach was needed. This came from an unexpected source.

In the fall of 1954 I was visited by Leo Szilard, the atomic physicist turned biologist and peacemaker (Lanouette, 1992), with whom I had worked as a research assistant in Chicago. Szilard was visiting his wife Trude Weiss, a physician at a Denver hospital and, unbeknownst to me at the time, he had been negotiating with Puck for a position at the University of Colorado. Szilard invited me to lunch at a restaurant within easy walking distance from the Health Center. We discussed my research project, and I had to admit it wasn't going that well. I explained that although there was no problem having cells replicate when large numbers of them were plated at high cell densities, we could only get a few percent of the cells to grow into colonies when they were dispersed as single cells. Szilard listened and then grew silent. I had learned while working in his

laboratory at the University of Chicago not to disturb such silences. He undoubtedly was involved in a creative thinking process he called "botching," a process best carried out without interruption.

After a few minutes, Szilard returned to the conversation with words to the effect, "Since cells grow with high efficiency when they have many neighbors, you should not let the single cells know they are alone." At that point I thought Szilard was about to invent a new field of science, the psychobiology of the single cell. What Szilard soon made clear was he meant the single cells should be grown in the same biochemical environment created when cells were grown in large numbers and high density. As envisioned by Szilard, the mass cultures of cells would provide the single cells with a constant supply of conditioned growth medium. I offered that the short half-life of any critical nutrient would be compensated for by its continuous generation from the large number of cells in the culture. Thus was born the "feeder cell" concept. We then turned to devising a practical way to achieve the desired conditions. On the proverbial restaurant paper napkin we laid out in schematic form a practical means to achieve the conditions Szilard sought. (I rue to this day my failure to preserve that napkin.) We decided to inoculate a large number of cells in the bottom of a 50-mm plate where they would tightly attach and spread to condition the medium continuously—the "feeder layer". The single cells would be physically separated from them on a plexiglas platform containing a piece of a microscope slide on which the single cells would be plated in microdrops of medium and incubated separately until they were firmly attached and spread out on the glass slide substrate. These slides would then be placed over the platform above the monolayer of cells already established from a large inoculum. Medium would be added so as to cover the glass slide which contained the attached single cells. All the conditions Szilard suggested would have been achieved. The single cells would not be alone, and they would be in the same biochemical environment, though physically separate from the mass of "feeder" cells. Truly an intelligent design.

After lunch I returned to the laboratory eager to share my excitement over this new approach with Puck. I was disheartened by his lack of enthusiasm for the proposal. Although my zeal for the experiment was dampened, it was not extinguished. As an eager new graduate student I found the experiment too compelling to ignore and sought out the department machinist, Bob Edgerton. Bob soon fabricated a U-shaped piece of plastic, which would hold the microscope slides to be inoculated with several microdrops of medium, each containing a single HeLa cell as verified microscopically.

I started the experiment on a Friday and verified that each of the several microdrops on the slide contained either one cell or none. I examined the slides microscopically one day later on Saturday and saw there were two cells in each location that had contained only one cell the previous day. Each of the single cells had doubled in number. A day later, on Sunday, all of the two-cell colonies now contained four cells! Szilard's concept had proved to be correct in practice. I phoned Szilard, and he and his wife Trude came to view the slides. Szilard beamed with delight. With trepidation I then phoned Puck and told him about the experiment. He joined us, looked at the physical setup, viewed the four-cell colonies, and left the laboratory without commenting. The cells in each clone continued to double in number for each of the next few days before the experiment was terminated because of contamination with mold. (The viewing was better with the lid removed from the petri dish.)

The plating efficiency was 100%, and there was no dilution error to contend with since the same cells or clones were observed on successive days, starting on day 0. Also, there was no evidence that cells from the monolayer were dislodged to the extent that they ended up contaminating the slides—not surprising in view of the fact that the combined height of the platform and slide put them over 200 cell diameters above the monolayer, and any detached cells, like those undergoing mitosis, would remain close to the monolayer due to gravity.

Days later when Puck returned to my laboratory after that historic Sunday, he announced that he had thought of a way to prevent the viable cells in the mass culture from dislodging from the monolayer and possibly contaminating the microscope slides. He said the feeder cells should be X-irradiated to destroy their reproductive capacity while retaining their metabolic activity for purposes of conditioning the medium. This turned out to be an important contribution to generating feeder cells. The first paper reporting the high plating efficiency of a mammalian cell appeared in 1955 (Puck and Marcus, 1955). It described the combined use of the plastic platform to separate the single cells from the feeder cell layer, as suggested by Szilard, and X-irradiation of the feeder cells as suggested by Puck. The report was later heralded as describing the procedure that made genetic analysis of somatic cells possible (Harris, 1995). Since the feeder cells generated after X-irradiation were reproductively sterile, though metabolically active, we soon thereafter plated the single cells directly onto the monolayer of feeder cells, thus dispensing with the use of the plastic platform. Improved formulations of growth media finally made it possible to dispense entirely with the X-rayed giant feeder cells for many lines and cultures of cells. Thus, by 1956 the composition and quality of the medium for growing HeLa cells had improved to the point that a feeder layer was no longer required to obtain 100% cloning efficiency. Nonetheless, feeder cells of various origins are still used to aid in the growth of fastidious cells like hybridomas and stem cells. Ted Puck had achieved his goal, and the genetics of somatic cells took a giant step forward.

Ironically, the X-ray machine that had stood unused for some three years since its arrival in the Department of Biophysics was turned on in 1954 and underwent a flurry of activity. The generation of monolayers of X-irradiated feeder cells became commonplace. Detailed characterization of the giant cells that constituted the feeder layers was reported (Puck and Marcus, 1956; Tolmach and Marcus, 1960), and the first survival curves generated from single cells were obtained following exposure to X-rays (Puck and Marcus, 1956), and to viruses (Marcus and Puck, 1958). Indeed, Ted Puck's goal had been accomplished.

The mid-1950s were an exciting time to be in Ted Puck's laboratory as the technology to drive somatic cell genetics became a reality (Harris, 1995). Puck's department also provided a stimulating intellectual environment with its fellow graduate students Steven J. Cieciura and Harold W. Fisher; postdoctoral fellows Masahiko Oda, Gordon Sato, and Richard Ham; faculty members Seymour Levine, Leonard Tolmach, Leonard Lerman, and John Cann; and a steady stream of visiting luminaries—Renato Dulbecco, James Watson, MacFarlane Burnet, and Linus Pauling come to mind. I still remember with great fondness the basement sessions in which we churned out large volumes of media from scratch—not surprisingly with Gordon Sato leading the way.

Postscript: In retrospect, the chronology of events that led to the historic achievement of the single-cell plating technique may ap-

pear confusing as gleaned from a footnote (#6) in its first report (Puck and Marcus, 1955). We stated, "In our earliest experiments test cells and feeder cells were placed in the same layer," and thanked Szilard for suggesting the arrangement in which the test cells were placed on top of the layer of feeder cells. I note that experiments in which the test cells and X-rayed feeder cells were placed in the same layer did not occur until *after* the use of the geometrical arrangement of cells involving the plastic support and microscope slides as described in the 1955 report—footnote #6 notwithstanding. In the interest of scientific accuracy and to aid the interested reader I describe these events in the order they actually occurred during my participation in them. First, the breakthrough concept of the feeder cell took place during the lunch time conversation between Szilard and myself as described above. Secondly, this was followed by the development of the X-rayed feeder cell—a hallmark contribution by Puck. Thirdly, the premier report on the use of feeder cells (Puck and Marcus, 1955) represents the original Szilard concept as it relates to the physical placement of the single cells on slides over a U-shaped plastic holder in which the viable feeder cells were replaced with X-rayed feeder cells as suggested by Puck. Coplating the test and feeder cells was first illustrated in a 1956 publication that described for the first time the sensitivity of human cells to X-rays (Puck and Marcus, 1956).

REFERENCES

- Fischer, A. Biology of tissue cells: essays. Cambridge University Press; 1946: 16–17.
- Gey, G. O.; Coffman, W. D.; Kubicek, M. T. Tissue culture studies on the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res.* 12:264–265; 1952.
- Harris, H. The cells of the body: a history of somatic cell genetics. Cold Spring Harbor Laboratory Press; 1995:47–51, 104–108.
- Lanouette, W. Genius in the shadows. New York: Charles Scribner's Sons; 1992:396–397.
- Marcus, P. I.; Puck, T. T. Host-cell interaction of animal viruses. I. Titration of cell-killing by viruses. *Virol.* 6:405–423; 1958.
- Puck, T. T.; Marcus, P. I. A rapid method for viable cell titration and clone production with HeLa cells in tissue culture: the use of X-irradiated cells to supply conditioning factors. *Proc. Natl. Acad. Sci. USA* 41:432–437; 1955.
- Puck, T. T.; Marcus, P. I. Action of X-rays on mammalian cells. *J. Exp. Med.* 103:653–666; 1956.
- Puck, T. T.; Robertson, O. H.; Lemon, H. M. The bactericidal action of propylene glycol vapor on microorganisms suspended in air. II. The influence of various factors on the activity of vapor. *J. Exp. Med.* 78:387–406; 1943.
- Sanford, K. K.; Earle, W. R.; Likely, G. D. The growth *in vitro* of single isolated cells. *J. Natl. Cancer Inst.* 9:229–246; 1948.
- Tolmach, L. J.; Marcus, P. I. Development of X-ray induced giant HeLa cells. *Exp. Cell Res.* 20:350–360; 1960.

FROM PHAGE GENETICS TO EARLY ANIMAL TISSUE CULTURE

Gordon H. Sato (Instructor, 1956–1958, Director Emeritus, W. Alton Jones Cell Science Center)

I had just finished graduate work at Cal Tech with Max Delbrück and a one-year postdoc with Gunther Stent and Niels Jerne at UC Berkeley when I entered Ted Puck's lab in Denver in 1956. How I got there is interesting. Two of the prominent workers in bacteriophage work, Renato Dulbecco and Ted Puck, were entering into animal cell work. Puck wanted to develop a plaque assay for animal viruses, but Dulbecco beat him to this so Puck decided to develop tissue culture work in the manner of *E. coli* technology, which had

been so productive. Along with Phil Marcus, he developed the single cell plating method and immediately used it to quantify the sensitivity of human cells to X-rays. At the time, tissue culture workers were an extremely narrowly specialized group, innocent of the advances being made in biochemistry and molecular biology. Ted thought that the field of tissue culture needed phage people with their habit of quantitative thinking and awareness of the broader issues of biology. He went to Max Delbrück and asked him to suggest possible candidates. I was the only one that Max thought he could spare from this talented group.

Upon entering the Puck group I found the transition from phage to tissue culture in full swing. The last phage student, Alan Garen, had just left to develop his own distinguished career. I found Leonard Lerman, a brilliant scientist, who had pioneered affinity chromatography as a graduate student with Linus Pauling. I worked with Harold Fisher on the nutrition of HeLa cells (Sato et al., 1957; Fisher et al., 1958, 1959). The more we purified one serum factor of interest, the bluer it got. This experience with ceruloplasm, a copper-containing protein, stood me in good stead for in later years I was attracted to transferrin, an iron-containing protein, which helped us develop hormonally defined serum-free media.

Good friendships were formed during this period: Steve Ciecura, Dmitri Markovin, Leonard Tolmach, and Howard Lee, who later became a psychiatrist with the California penal system. Later, Dick Ham joined the group, and his meticulous work with nutrition was a great help to me.

I remember Ted Puck for his actions toward Yuhin Tjio, which reveal a side of Puck that is little commented on. Tjio was a very talented cytologist who discovered the diploid number of human chromosomes was 46 (Tjio and Levan, 1956). Previously, this number was thought to be 48. Puck immediately understood the importance of this and brought him from Sweden to Denver. Tjio's life had been unspeakably horrible from childhood, and he was chronically depressed. As a young adult in Japanese-occupied Indonesia, he helped Dutch prisoners by sneaking in medicines into the prison camp. He was caught and escaped. He then tried to take medicine to his Dutch friends again and was captured. He narrowly escaped execution with the end of the war. For his heroic exploits he was decorated by the Dutch government. When he made his pivotal discovery, he did not have an advanced degree and was treated as an unimportant lackey. Puck arranged for him to receive a Ph.D. from the University of Colorado, and obtained a position for him at the NIH. What had been a terrible life was now made bearable. With Puck, Tjio described all the human chromosomes (Tjio and Puck, 1958) and this resulted in the Denver classification system.

For my part, I am grateful to Ted Puck for introducing me to a field that has been the greater part of my professional life.

REFERENCES

- Fisher, H. W.; Puck, T. T.; Sato, G. H. Molecular growth requirements of single mammalian cells: the action of fetuin in promoting cell attachment to glass. *Proc. Natl. Acad. Sci. USA* 44:4–10; 1958.
- Fisher, H. W.; Puck, T. T.; Sato, G. H. Molecular growth requirements of single mammalian cells. III. Quantitative colonial growth of single S3 cells in a medium containing synthetic small molecular constituents and two purified protein fractions. *J. Exp. Med.* 109:649–661; 1959.
- Sato, G. H.; Fisher, H.; Puck, T. T. Molecular growth requirements of single mammalian cells. *Science* 126:961–964; 1957.
- Tjio, J. H.; Levan, A. The chromosome number in man. *Hereditas* 42:1–6; 1956.

Tjio, J. H.; Puck, T.T. The somatic chromosomes of man. *Proc. Natl. Acad. Sci. USA* 44:1229–1237; 1958.

OPTIMIZED MEDIA: SETTING THE STAGE FOR GENETIC STUDIES

Richard G. Ham (Postdoctoral Fellow and Assistant Professor, 1958–1965)

I first encountered Theodore T. Puck when he presented a seminar at the University of Texas. My doctoral research there was an attempt to identify biochemical mechanisms involved in cellular differentiation in a simple model system, head regeneration in *Hydra*. These studies had convinced me that even the simplest of multicellular model systems were far too complex and that I needed to be able to work with isolated cells. Ted's single cell culture system seemed like a perfect fit. I eagerly accepted his offer of a postdoctoral position.

When I joined Ted's research team in 1958, single cell techniques were already well developed and used in a variety of applications, including precise measurement of radiation sensitivity of isolated cells (reviewed by Marcus, 2006). Ted was looking increasingly toward his long-term goal of using cultured cells for genetic studies (Puck, 1994). The HeLa cell line was not suitable for such studies because of its chromosomal abnormalities. Cultures of euploid human cells had been successfully established, as well as cultures of Chinese hamster cells, which were of special interest because of their low chromosome number ($2n = 22$) (Puck et al., 1958).

The value of cultured cells for human karyotypic analysis had already been demonstrated and a system for numbering human chromosomes had been proposed (Tjio and Puck, 1958). Alternative numbering systems were also being proposed. Ted considered it critically important for everyone to use the same number assignments, even if they had to be drawn from a hat. Soon after my arrival, he played a major role in bringing everyone who had published a human karyotype to Denver to adopt a uniform system of numbering. Four days were required to achieve consensus, but the resultant numbering system (Robinson et al., 1960) became the standard that everyone now uses. In a retrospective article, Ted describes the last minute scramble to fund that conference after an initial application was rejected (Puck, 1994).

At the time of my arrival, Harold Fisher and Gordon Sato had recently replaced the serum requirement for growth of HeLa S3–9 cells with two purified protein fractions, fetuin and serum albumin (Fisher et al., 1959). However, growth of the more fastidious euploid human and Chinese hamster cells continued to require serum. Although my thesis had a different focus, nutritional biochemistry was heavily emphasized in my graduate coursework. Ted therefore suggested that developing a nutrient medium for clonal growth of a diploid Chinese hamster ovary (CHO) cell line with fetuin and albumin would be an appropriate postdoctoral project.

The CHO cells initially had a fibroblast-like morphology. I therefore used the term F1 (fibroblast nutrients 1) to describe the nutrient mixture (40% N16, 4% NCTC109, 56% Saline F) that was used for their growth with 15% serum. Early tests showed that growth could be improved by diluting the N16 to 20%, and that the most critical component of NCTC 109 was cysteine. Adding new components, including pyruvate, serine, thymidine, and iron, and adjusting calcium, cysteine, and folic acid to optimum concentrations further improved clonal growth and reduced the requirement for serum proteins. The resultant nutrient mixture, designated F7, supported

clonal growth of CHO cells with 0.5 mg/ml human serum albumin and 1.0 mg/ml fetuin (Ham, 1962).

During this time period, the Tissue Culture Association did not fully recognize the value of the radical new single cell plating technique that Ted and his colleagues had developed. Instead, they continued to emphasize classical methods in their training courses. To counter this, Ted organized his own course, which he offered for several years in Estes Park, Colorado. As my work with the CHO cells progressed, one of my additional duties was to coordinate the preparation of instructional materials for that course. This ultimately led to publication of a modified version of the course manual in *Methods in Enzymology* (Ham and Puck, 1962). This experience helped set the stage for a number of long reviews and methods papers that I have written since then.

The studies that led to development of F7 clearly demonstrated the importance of precise nutrient balance. A full round of optimization of all nutrient concentrations generated nutrient mixture F10 (Ham, 1963a), which supported clonal growth of CHO with much lower levels of fetuin and albumin.

Although Ted liked to keep each member of his research team narrowly focused on a specific research project, I began to ask what the real functions of fetuin and albumin were. Serum albumin is notorious for binding everything imaginable. For CHO cells, linoleic acid, critically adjusted to a very narrow optimum concentration, quite effectively replaced albumin in F10 plus fetuin (Ham, 1963b). One of the known roles of fetuin was to serve as a trypsin inhibitor. The first step in its replacement was a very mild trypsinization procedure that left very little residual trypsin in the clonal cultures. With that problem solved, the remaining need for fetuin could be replaced by putrescine or related polyamines (Ham, 1964).

A final round of optimization of nutrient levels in the absence of any added proteins was then performed with CHO cells and with a male Chinese hamster lung line (CHL). These studies produced medium F12, which supported more rapid clonal growth in the absence of added proteins and could also be used for extended serial culture (Ham, 1965). A slight modification, F12M, with a lower level of zinc, also supported clonal growth of the classic mouse L-929 cell line.

Thus, the requirement for added macromolecules was completely eliminated by providing all of the nutrients needed by the cells and by optimizing all of their concentrations. In retrospect, however, it is now evident that the cell lines had undergone subtle transformations that enabled their protein-free growth. Among other things, the morphology of the CHO cells gradually changed from fibroblast-like to more epithelial. In addition, although their chromosome number remained near diploid, they became distinctly aneuploid over time.

A few years later, Ted's lab was able to achieve "reverse transformation" of CHO cells, including reversion to a fibroblast-like morphology by treatment with cyclic AMP or testosterone (Hsie and Puck, 1971). I am not aware of comparable studies on the CHL cells, but it appears likely that they also underwent a similar transformation. Under most circumstances, growth of nontransformed normal cells continues to be dependent on externally supplied macromolecular growth-promoting substances.

Comparison of the nutrient requirements of CHO and CHL cells revealed an unexpected difference that gave a major boost to Ted's long-term goal of using cultured cells for genetic studies. The CHO line had always been grown in a proline-containing medium and

required proline for growth. However, the CHL cells did not require proline, which was generally considered by other investigators to be a "nonessential" amino acid.

By this time there were claims that DNA-mediated genetic transformation could be achieved with cultured cells (Szybalska and Szybalski, 1962). The proline-dependent CHO cells in combination with the proline-independent CHL cells seemed like the perfect system for a definitive test. When CHO cells that had been exposed to DNA from CHL cells were plated at high density into proline-free F12, a few proline-independent colonies grew out. However, similar numbers of colonies also appeared in the control with comparable numbers of untreated CHO cells.

Thus, transformation was not demonstrated. However, the control revealed that the proline-dependent cells carried an auxotrophic mutation with a low level of reversion. The revertants had not been detected in clonal assays, but were easily seen with the larger inoculum in the transformation experiment. The CHO line had been subcloned repeatedly to maintain a diploid (or near diploid) state. In retrospect, it appears that a proline-dependent mutant was fortuitously selected during one of the clonings.

At this point, I was ready to depart to a new position in Boulder. However, follow-up studies on this mutation (Kao and Puck, 1967) marked the beginning of a new phase of studies on somatic cell genetics in Ted's laboratory (reviewed by Patterson, 2006). In addition, a new subclone of the proline-dependent CHO line, CHO-K1 was generated, which has subsequently become a widely used standard in biotechnology.

After I moved to Boulder, I was unable for many years to grow CHO or CHL cells in protein-free medium F12. We finally found that the cells had a requirement for selenium (Hamilton and Ham, 1977), which was apparently present as a contaminant in the chemicals or water in Ted's lab. Based on current knowledge of trace element requirements of whole animals, it appears likely that numerous other trace elements will also be needed for growth of cultured cells in media that are truly free of trace element contamination.

Ted's quest for definition of cellular growth requirements continued to influence research of his former associates for many years. Based on protein-free growth of CHO cells, my laboratory vigorously pursued a similar goal for diploid human cells through medium optimization (Ham and McKeehan, 1979). Although we greatly reduced the serum protein requirement, we could not eliminate it completely. Gordon Sato and his colleagues were able to use mixtures of hormones and growth factors to replace serum requirements for many different types of permanent cell lines (reviewed by Barnes and Sato, 1980; Sato, 2006). This approach worked well for transformed cell lines, but not for normal cells. Finally, graduate students and postdocs from our two labs got together and urged each lab to look at what was happening in the other. Bringing these two aspects of Ted's original goals back together led to substantial progress in both labs.

Finally, I would like to close with a special word of appreciation. During the entire seven years that I was in Ted's laboratory, first as a postdoctoral, and then as an assistant professor, all of my research was totally supported by his research grants. However, when the time came to publish my studies on growth requirements, he proposed that they be published with my name as sole author. His generosity gave my research career a huge boost. For years afterward, when other investigators met me for the first time, they com-

mented that they had expected to see someone much older. Unfortunately, that is no longer true, but for the record, thank you, Ted.

REFERENCES

- Barnes, D.; Sato, G. H. Methods for growth of cultured cells in serum-free medium. *Anal. Biochem.* 102:255–270; 1980.
- Fisher, H. W.; Sato, G. H.; Puck, T. T. Molecular growth requirements of single mammalian cells III. Quantitative colonial growth of single S3 cells in a medium containing synthetic small molecular constituents and two purified protein fractions. *J. Exp. Med.* 109:649–661; 1959.
- Ham, R. G. Clonal growth of diploid Chinese hamster cells in a synthetic medium supplemented with purified protein fractions. *Exp. Cell Res.* 28:489–500; 1962.
- Ham, R. G. An improved nutrient solution for diploid Chinese hamster and human cell lines. *Exp. Cell Res.* 29:515–526; 1963a.
- Ham, R. G. Albumin replacement by fatty acids in clonal growth of mammalian cells. *Science* 140:802–803; 1963b.
- Ham, R. G. Putrescine and related amines as growth factors for a mammalian cell line. *Biochem. Biophys. Res. Commun.* 14:34–8; 1964.
- Ham, R. G. Clonal growth of mammalian cells in a chemically defined, synthetic medium. *Proc. Natl. Acad. Sci. USA* 53:288–293; 1965.
- Ham, R. G.; McKeehan, W. L. Media and growth requirements. *Meth. Enzymol.* 58:44–93; 1979.
- Ham, R. G.; Puck, T. T. Quantitative colonial growth of isolated mammalian cells. *Meth. Enzymol.* 5:90–119; 1962.
- Hamilton, W. G.; Ham, R. G. Clonal growth of Chinese hamster cell lines in protein-free media. *In Vitro* 13:537–547; 1977.
- Hsie, A. W.; Puck, T. T. Morphological transformation of Chinese hamster cells by dibutyl adenosine cyclic 3',5'-monophosphate and testosterone. *Proc. Natl. Acad. Sci. USA* 68:358–361; 1971.
- Kao, F.-T.; Puck, T. T. Genetics of somatic mammalian cells IV. Properties of Chinese hamster cell mutants with respect to the requirement for proline. *Genetics* 55:513–524; 1967.
- Marcus, P. I. The genesis of feeder cells: concepts and practice. *In Vitro Cell. Dev. Biol.* This issue, 2006.
- Patterson, D. Ted Puck and the era of somatic cell genetics. *In Vitro Cell. Dev. Biol.*, this issue; 2006.
- Puck, T. T. Living history biography. *Am. J. Med. Genet.* 53:274–284; 1994.
- Puck, T. T.; Cieciura, S. J.; Robinson, A. Genetics of somatic mammalian cells III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. Med.* 108:945–959; 1958.
- Robinson, A.; Book, J. E.; Chu, E. H. Y., et al. A proposed standard system for nomenclature of human mitotic chromosomes. *JAMA* 174:159–162; 1960.
- Sato, G. H. From phage genetics to early animal tissue culture. *In Vitro Cell Dev. Biol.*, this issue; 2006.
- Szybalska, E. H.; Szybalski, W. Genetics of human cell lines. IV. DNA-mediated heritable transformation of a biochemical trait. *Proc. Natl. Acad. Sci. USA* 48:2026–2034; 1962.
- Tjio, J. H.; Puck, T.T. The somatic chromosomes of man. *Proc. Natl. Acad. Sci. USA* 44:1229–1237; 1958.

TED PUCK AND THE ERA OF SOMATIC CELL GENETICS

David Patterson (Postdoctoral Fellow through Professor, 1971–2005)

It was my fortune to know Ted Puck for almost 35 years, having arrived in Denver in 1971 to work with him as a postdoctoral colleague. I first heard Ted speak at Harvard University in the late 1960s when I was a graduate student at Brandeis University studying temperature sensitive mutants of *E. coli*. At this time, I had already met Gordon Sato, who taught a section of the biochemistry class at Brandeis. I was already hooked on genetics and thought that the development of somatic cell genetics in Ted's lab was the wave of the future. In 1969, I applied to join him as a postdoctoral Student and was fortunate enough to be accepted. It was a good thing, since my recollection is that I didn't apply anywhere else.

When I joined Ted in the fall of 1971, he and Dr. Fa-Ten (Louie)

Kao had already developed the BudR-Visible Light procedure for isolating auxotrophic mutants of CHO-K1 cells (Kao and Puck, 1968). My initial idea was to apply this method to isolate temperature-sensitive mutants of CHO-K1 cells, and I was fortunate enough to receive a Damon-Runyon Cancer Research Foundation fellowship for this work. Eventually, the work proved successful (Patterson et al., 1976), but it soon became apparent to me that this was probably not an optimal strategy for studying the somatic cell genetics of cancer, which was the impetus for my joining the laboratory. During those early years, Ted and his colleagues Louie Kao, Larry Chasin, and others were developing crucial genetic methods of somatic cell genetics, most notably the use of various mutagens to induce mutations, somatic cell hybridization for complementation analysis of mutants defective in the same biochemical pathway, and mapping genes to human chromosomes (Kao et al., 1969; Kao and Puck, 1972). Ted encouraged me to take part in this ongoing endeavor, which was a turning point in my career (Patterson et al., 1974).

Ted demanded rigor, dedication, independence, and curiosity. As part of my training, he strongly urged me to audit Dr. Art Robinson's medical genetics class, which I did not once, but twice. It was there that I first learned about Down syndrome. Study of this syndrome has been a major part of my life's work. It came about because of Ted's suggestion.

As others have also mentioned, Ted helped my career in other, more subtle ways. He was always eager to discuss ideas and results, and invariably had important insights and suggestions. He very generously supported my work from his own grant funds and funds that he was able to raise from private sources. In spite of this, he insisted that I publish my results independently. He requested that I organize the Institute's seminar series, which involved inviting speakers and hosting them on their visits. Because of this, I was able to personally interact with some of the leading scientists of the day, including Francis Crick, Fred Sanger, Max Perutz, Barry Blumberg, Paul Berg, Marshall Nirenberg, Janet Rowley, Ruth Sager, and many others. Of course, Ted suggested most of the people to invite, and they were his friends and acquaintances. These interactions were a source of great inspiration as well as scientific insight.

Ted was deeply concerned about the teaching of science, and indeed about teaching in general, from elementary school to the postgraduate level. He was a member of the Paideia Group, organized by philosopher Mortimer Adler, which considered the state of education in the United States, and in 1984 published The Paideia Program, an Educational Syllabus. The ideas expressed by this group are still influential in education today. He was a member of the Editorial Board of the Encyclopedia Britannica. I remember that very often Ted and I would meet to discuss a scientific issue and soon we would be talking about the state of medical school curricula and the changes that needed to be made to make sure that physicians would be appropriately trained for what he considered to be the ongoing revolution in medical knowledge and practice. We continued to discuss his new ideas on the state of science and medical education in the weeks before his death.

For the last few years of his life, Ted's major research focus was on prevention of cancer and other genetic diseases. To this end, he devised sensitive and practical tests to screen for environmental agents that could cause mutations and cancer. He worked towards this goal tirelessly until the very end of his life. Only a few days before his death, Ted, Dr. Sharon Graw, and I began a new collab-

oration to extend his research by taking advantage of some of the most modern molecular biological and bioinformatics techniques. Ted hoped that this method would lead to ways to detect these dangerous compounds at extremely low levels so that people could be protected from them. This project is completely consistent with his early successful efforts to find the true lethal dose of radiation and to warn of its harmful effects. We plan to continue this important research.

On a personal level, Ted was an inspiration to me. He was by far the most influential figure in my own scientific career, as he was for many others. He was a great friend and role model. I was honored to be his friend and colleague. His death is a great loss, but his life and friendship were great gifts. He will be missed, and his memory cherished.

REFERENCES

- Kao, F. T.; Chasin, L.; Puck, T. T. Genetics of somatic mammalian cells. X. Complementation analysis of glycine-requiring mutants. *Proc. Natl. Acad. Sci. USA* 64:1284–1291; 1969.
- Kao, F. T.; Puck, T. T. Genetics of somatic mammalian cells. VII. Induction and isolation of nutritional mutants in Chinese hamster cells. *Proc. Natl. Acad. Sci. USA* 60:1275–1281; 1968.
- Kao, F. T.; Puck, T. T. Genetics of somatic mammalian cells: demonstration of a human esterase activator gene linked to the adeB gene. *Proc. Natl. Acad. Sci. USA* 69:3273–3277; 1972.
- Patterson, D.; Kao, F. T.; Puck, T. T. Genetics of somatic mammalian cells: biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism. *Proc. Natl. Acad. Sci. USA* 71:2057–2061; 1974.
- Patterson, D.; Waldren, C. A.; Walker, C. Isolation and characterization of temperature-sensitive Chinese hamster ovary cells after treatment with UV and X-irradiation. *Somatic Cell Genet.* 2:113–123; 1976.