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MIN CHUEH CHANG

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A Biographical Memoir by

ROY O. GREEP

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BY ROY O. GREEP

THE LIFE WORK OF Min Chueh Chang centered on a discrete portion of the mammalian reproduction process, the part that begins with the existence of male and female free living gametes and ends with their successful union, fertilization. In this sphere Chang was a world leader, a giant of his time. That, however, is only part of the fame that is conjured up by the mention of his name. Actually, Chang is best known in the public mind for his work on the development of the oral contraceptive, "the Pill." The latter benefited millions of women and fomented a social/sexual revolution. This freeing of the sexual act from the threat of conception led to major changes in the way men and women live together.

To bring these two related but very different aspects of Chang's research into perspective, it is important to note that of his forty-five years in research only five (1951 through 1956) were spent in proving the effectiveness of certain steroids in controlling fertility in laboratory mammals when administered orally. This was his greatest contribution in pragmatic terms.

This brief departure from Chang's abiding interest in eggs and sperm themselves is in keeping with his recognition of the critical need for better means of controlling

human fertility. It is noteworthy that Chang's work on developing the Pill utilized fundamental information already available. Except for the mode of administering the contraceptive steroids, little was added to existing knowledge. On the contrary, Chang's monumental work on fertilization was purely an exercise in basic science for the purpose of gaining new insight into the mechanism of fertilization. That this turned out to have great practical significance was, of course, a personal satisfaction, but it was the plaudits of the scientific community that pleased him most.

His life career is a story of triumph and disappointments, perseverance and major accomplishments, accolades, international recognition, and, lastly, an element of what Peter Medawar recognized as chance. It was largely by chance that Chang often found himself the right man at the right place at the right time. This was especially true at Cambridge University (1939-45) and again at the Worcester Foundation for Experimental Biology (1951-56). Chang was aware of these favoring circumstances. In an unpublished manuscript titled "Reminiscences on the Study of Animal Reproduction and Association with Reproductive Biologists," Chang wrote extensively, forthrightly, and illuminatingly on the many preceptors and counselors to whom he was greatly indebted for their material help, guidance, and encouragement.

Lastly, note need be made of the fact that Chang grew up, as it were, with a newly founded institution that provided him with the opportunity and the facilities to carry out his extended program of research and to attain preeminence in the world of science. Chang returned this favor by leaving to the Worcester Foundation for Experimental Biology a proud legacy of prestige and renown.

Not the least of what made Chang a notable and endearing character is that he was every inch a kind, generous, fair-minded, and gentle person whose integrity was a given.

PERSONAL HISTORY

Min Chueh Chang was born in Tai Yuan (Shanxi province), China, on October 10, 1908. His father, a magistrate, was able to provide him with a quality education, including in 1933, a bachelor's degree in animal psychology from Tsing Hua University in Peking. Over the next few years of turbulent times in China, Chang stayed at the university as a teacher and made some original observations on the staining of nerve cells that gained publication in a prestigious American journal.

Chang's brilliance of mind and unbounded curiosity did not go unnoticed. In 1938 he was encouraged to compete in a national examination for a few much-prized fellowships to study abroad and he won. He opted for a year of study in agricultural science at Edinburgh University. At year's end the chilly climate and his perception of some bias against foreigners were not to his liking. An appealing invitation from Arthur Walton to join him in research on ram spermatozoa at Cambridge University was gladly accepted. This was in keeping with Chang's newfound interest in reproductive biology, a departure from his initial intent on a career in behavioral psychology. There under Walton's tutelage and association with such other greats as Sir John Hammond and F. H. A. Marshall, Chang became engrossed in research. On the basis of his multiple observations on the effect of testicular cooling and various hormonal treatments on the respiration, metabolism, and survival of sperm in rabbits and some farm animals, Chang was awarded a Ph.D. degree in animal breeding by Cambridge University in 1961.

The options available to Chang at that time included returning to China and sharing in the suffering of his parents and friends. Fortunately, counselors at Cambridge pre-

vailed upon him to remain there. With the exigencies imposed by World War II, the best that could be provided was maintenance support and limited opportunities for research.

At war's end Chang again was torn between returning to China or finding elsewhere an outlet for his study of fertility. He sought and was granted a one-year fellowship with Gregory Pincus to learn the technique of in vitro fertilization before returning home. At the time of Chang's arrival in the United States, Pincus was at Clark University with Hudson Hoagland, and the two of them were in the process of founding the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, just outside Worcester. Chang was given a room at the newly created foundation, and he often told with some delight how he served as night watchman. It was soon evident to Pincus and Chang that they were an effective team with many common interests in the broad field of reproductive biology. Moreover, they almost immediately formed a warm, personal, and enduring relationship. It was there that Chang would spend the remainder of his illustrious and rewarding career in research on matters relating to mammalian fertility.

As funds for support of research on reproduction became increasingly available after mid-century, Chang's laboratory began to attract a cadre of highly competent young investigators who today are distinguished leaders in basic and clinical research on reproduction. In Chang's laboratory they were mainly left to their own devices except that Chang was always at hand for helpful guidance and advice when needed. Among the group of approximately 100 fellows and associates, singling out any for mention risks the sin of omission, but mention of a few will illustrate the distinction of the group as a whole: J. M. Bedford, C. R. Austin, R. Yanagimachi, M. R. J. Harper, Y. Toyodo, R. H. F. Hunter, J. H. Marston, T. Iwamatsu, and H. Miyamoto. With

this concentration of expertise in Chang's special field, his laboratory became an international crossroad. An unending influx of distinguished visitors was a significant factor in establishing the Worcester Foundation for Experimental Biology as an important biomedical research center.

Chang's work habits were incredible. He personified what is dubbed a workaholic. His quest for better understanding of the intricate series of sequential physiological mechanisms involved in the fertilization of mammalian ova was the dominant and consuming factor in his life. Some measure of the intensity of his labors will be evident from the fact that at the peak of his productivity he was publishing up to nineteen papers annually—all in first-rate, peer-reviewed journals and all reporting substantive findings. Chang was by his own admission a patient and persevering type of investigator. He had long-range goals toward which he planned his experimentation assiduously. The strong likelihood of gaining substantive new information from each carefully designed experiment was a contributing factor to his prolific productivity. Chang's bibliography lists 347 papers, of which he was sole author of 112 and senior author of another 38. Most scientists will agree that such prodigious effort comes at the expense of time with the family, cultural pursuits, and reflections on broader issues within and outside science.

Shortly after Chang arrived in the United States he married an American-born Chinese woman, Isabelle Chin, whom he met by chance in the Yale University library. Their three children include two daughters, Claudia Chang Tourtellotte, head of the anthropology department at Sweet Briar College in Sweet Briar, Virginia; Pamela O'Malley Chang, an architect and civil engineer in San Francisco, California; and a son, Francis Hugh Chang, director of a health center in Boston, Massachusetts.

Chang was neither a family man in the usual sense nor a doting father. In his private life he was a Confucian scholar and held to the principles of strict discipline for himself and his son and male dominance of the marital relationship. Much credit must be given to Chang's talented wife for her willing acquiescence in the role of a Confucian wife as her part in enabling Chang to develop his full potential unhindered by domestic concerns. On Chang's behalf it can be said that he followed the cultural traditions of his Asian background in a Western setting yet retained the profound respect of his family.

In his later years Chang traveled extensively to many parts of the world to participate in meetings devoted to his special field of investigation. Such attendance was almost always as an invited speaker. His distinguished accomplishments were otherwise recognized by numerous honors and awards. A partial list includes the Albert Lasker Award (1954), Ortho Medal and Award by the American Fertility Society (1961), Hartman Award by the Society for the Study of Fertility (1971), Frances Amory Prize by the American Academy of Arts and Sciences (1975), Wippman Scientific Research Award by the Planned Parenthood Federation of America (1987), and election to membership in the National Academy of Sciences in 1990.

PROFESSIONAL HISTORY

A detailed account of Chang's experimental work as depicted in nearly 350 publications is far beyond accommodation here. A look at some of his major accomplishments must suffice. Chang's life work involved a series of highly interrelated projects. The first dealt with the metabolism, motility, and fertilizing capacity of ram sperm. This was closely tied to a concurrent attempt to improve the effectiveness of artificial insemination in farm animals. It being

wartime this had the prospect of increasing food production. Once the war ended and Chang had moved to the United States, he was able to take up a quest that he had had in mind for some time—namely, fertilization of ova outside the mammalian body (in vitro fertilization). To that end he sought first to understand why sperm from the epididymis or ejaculate were motile but incapable of penetrating ova.

Chang's competence in reproductive biology was occasioned by having to understand, and to manipulate, the reproductive status of the host animals from which he obtained male and female gametes. It was with this background that he was eminently qualified to meet the challenge of evaluating, on a virtually emergency basis, a wide range of steroidal compounds as potential orally active antifertility agents in the early 1950s.

In his initial studies on eggs and sperm Chang carried out a variety of experiments mainly to acquire expertise in the techniques involved and to gain a thorough knowledge of the field. He examined the motility and fertilizing capacity of sperm taken from different areas of the male reproductive tract, with special attention to sperm from various parts of the epididymis. Out of this came the finding that cooling by simply applying ice to the scrotum caused severe disintegration of sperm from the lower end of the epididymis. This is now a generally recognized phenomenon known as cold shock. It occurs in a critical range of temperatures (13°-0°C) and results in a breakdown of membrane structure and function. Chang showed that sperm subjected to deep freezing must be protected by a cryoprotective agent found in egg yolk. Chang's original observation on cooling led to a massive study of cold shock. Obversely, Chang found that exposure of unfertilized rabbit ova to elevated temperatures destroyed their fertilizability.

Early in his career Chang was intrigued by the prodigality of sperm production and made several observations on the effect of the number of sperm on fertilization of ova. He once estimated that the human male produces about 1 billion sperm for every egg released by the female gonads. He found that of approximately 200 million sperm deposited in the rabbit vagina by ejaculation or artificial insemination barely 1 percent make it past the cervical barrier to the uterine cavity and only about 5,000 find their way past the utero-tubal junction. Fewer still reach the site of fertilization in the outer segment of the oviduct.

Earlier literature claimed that fertilization required the presence of what were termed swarms of sperm in contradistinction to vanguards. The belief was that large numbers of sperm were necessary to release a lytic agent that would dispel the follicular cells surrounding the oocyte, the cumulus oophorus, and corona radiata. Chang showed that it is the physiological integrity of an individual sperm that is important for fertilization. He also found that a single sperm can penetrate the cumulus mass of cells and reach the zona pellucida, a thick mucoprotein membrane enveloping the ovum. Each sperm head carries an attached packet, the acrosome, containing hyaluronidase, which is released by the acrosome at the site of fertilization and was believed to effect the dispersal of cumulus cells. Chang found that the number of sperm at the site was far too few to accomplish this event. Adding hyaluronidase to sperm suspensions did not prove to be beneficial. Chang also disproved an alleged claim that phosphorylated hesperidin, a hyaluronidase inhibitor, had an antifertility action when administered orally.

Since large numbers of sperm are of no benefit to fertilization, their production in astronomical numbers throughout reproductive life posed a challenge to Chang. He posited that every population of sperm is comprised of some

that are strong and others weak, morphologically defective, or aged. Since only the strongest of the strong reach the site of fertilization, Chang held that the more sperm entering the female reproductive tract the more this would provide for greater variation in the recombination of genes.

Chang also did a large amount of work on the local milieu of sperm. He wanted to understand the effect of the very different environmental factors to which sperm are exposed during their passage through the epididymis and ascent of the female reproductive tract. The fertilizing capacity of rabbit epididymal sperm was not benefited by suspension in rabbit seminal plasma as compared to Ringer's or Tyrode's solution. On the matter of osmolality, Chang and Thorsteinsson found that rabbit sperm could tolerate without ill effect on either motility or fertility half the strength or twice the strength of Ringer's solution at neutral pH. They also found that sperm could survive a wide range of pH at isotonicity—namely, from 5.57 to 10.94! That is fortuitous since sperm often encounter a wide range of pH in the human vagina.

Sperm deposited in the rabbit vagina on mating reach the fallopian tubes within minutes and await the arrival of ova for fertilization ten to twelve hours later. In a fateful experiment Chang deposited ejaculated sperm in the tubes to coincide with the arrival of ova. Fertilization failed. Testing his speculation that the waiting period was the crucial factor, Chang next deposited sperm in the tubes six to eight hours before the arrival of ova and obtained fertilization. This finding that sperm must undergo an incubation period in the female reproductive tract before they acquire fertilizing capacity was independently reported in 1951 by Chang and his close friend and arch rival, C. A. Austin of Australia. For both investigators this was at once a blessing by virtue of immediate confirmation and inescapably some

sense of disappointment. A year later Austin named this phenomenon sperm capacitation—a term now in wide use by reproductive biologists and clinicians.

Chang and his associates proceeded to show that capacitation was a general phenomenon occurring among all mammalian species studied. They found that the duration of the waiting period varied somewhat among species: rabbit, five to six hours; rat, four to five hours; mouse, one hour; golden hamster, two to three hours; and sheep, one and one-half hours. They also found that sperm could acquire capacitation in the uterus as well as the tubes. An exciting extension of capacitation came with the discovery by Chang in 1957 that capacitated sperm exposed to either seminal plasma or blood serum from the same species or from other species lost their capacitation, an event termed decapacitation. This factor was found by Bedford and Chang to be a high-molecular-weight substance that adheres to the surface of sperm and is removable by centrifugation at 105,000 times g. Taking this one step further, they found that decapacitated sperm could be recapacitated by placing them back in the uterus or tubes.

In a 1958 study of the possible influence of the hormonal status of the female rabbit reproductive tract on the capacitation of sperm, Chang found that ejaculated sperm placed in the uteri of pseudopregnant or progesterone-treated rabbits failed to become capacitated. In striking contrast, sperm placed in the fallopian tubes of these rabbits did become capacitated. Capacitation was also achieved in the uteri of immature or ovariectomized rabbits with or without estrogen treatment.

Chang's mastery of capacitation did not prove to be the Holy Grail. He could not have been unaware that by the discovery of capacitation he was one step closer to the achievement of in vitro fertilization. Back in 1945 Chang's main

purpose in coming to the Worcester Foundation was to learn the technique of in vitro fertilization from Gregory Pincus. As early as 1935 Pincus claimed to have obtained living young from rabbit eggs fertilized in vitro and returned to the doe. Doubts as to the authenticity of this report lingered, and Chang working in Pincus's laboratory was not able to repeat those findings. This opened an intense and competitive search for a solution to this important problem. In 1954 Thibault and associates reported early embryonic development in eggs fertilized in vitro. Chang's crowning achievement came in 1959 with his demonstration that eggs from a black rabbit fertilized in vitro by capacitated sperm from a black male and transferred to a white female resulted in the birth of a litter of black young. This evidence seemed beyond question, and was, but some skepticism persisted for a while.

The circumstances that allowed in vitro fertilization in rabbits proved to be species specific. For many years, Chang and his students continued to define the varying specific conditions required for in vitro fertilization in several species. To wit, in 1963, Yanagimachi and Chang reported the first successful fertilization of golden hamster eggs in vitro. They used Tyrode's solution, containing glycine and sperm capacitated in the uterus. Next came the in vitro fertilization of mouse ova incubated in bovine follicular fluid, as reported by Iwamatsu and Chang in 1969, and in the same year Pickworth and Chang succeeded in fertilizing Chinese hamster eggs in vitro. In 1973-74 Miyamoto and Chang and Toyoda and Chang reported fertilization of rat eggs in vitro, and in 1978 Hanoda et al. accomplished the same for deermouse eggs.

In an extension of this study of in vitro fertilization, Yanagimachi and Chang (1963) found penetration of rabbit ova by sperm taken from the epididymis, thus showing

that capacitation had occurred in vitro. In 1973 Miyamoto and Chang observed fertilization of mouse eggs by in vitro capacitated sperm, and a year later Toyodo and Chang made similar observations on rat sperm capacitated in a chemically defined medium.

It was on the basis of this animal data that Steptoe, Bravister, and Edwards were able to achieve in vitro fertilization of human ova. Later, the landmark birth on July 25, 1978, of the world's first test-tube baby, Louise Joy Brown, in Oldham, England, was recorded by Steptoe and Edwards.

From this overview of the entire spectrum of Chang's investigational program, it is evident that the central and constant objective was understanding the detailed circumstances involved in the process of sperm penetration and fertilization of mammalian ova. Such was the fabric of his illustrious career.

In the twilight of his career Chang became disturbed by the confusion and controversy appearing in the literature as a result of disagreement as to what changes are to be considered part of the capacitation process. Capacitation as originally defined both by Austin and Chang in 1951 included all the changes that enable sperm to penetrate eggs. Over the years a variety of structural and chemical changes in the sperm during the capacitation were described, some being considered as components of the capacitation process, others not. The bulk of the controversy centered on whether the acrosome reaction was a separate feature or a part of capacitation. Among those holding that capacitation was simply a preliminary that enabled the acrosome reaction to occur were several of Chang's former colleagues, most notably Bedford, Austin, and Yanagimachi. In a review article on capacitation published in 1984, Chang argued forcefully at some length as to why the original definition, which includes all the changes that enable a sperm to pen-

etrate the egg, should be retained. He dealt strictly with the facts on a totally impersonal basis. It was an elegant appeal to await full understanding of what happens to sperm during that still ever so puzzling waiting period.

PERSONAL COMMENTS BY THE AUTHOR

Chang was an easily recognized figure, tall and slimly built with a copious head of dark hair tinged with gray. His twinkling eyes and ready smile were prominent features of his friendly greetings. Despite being a man of illustrious international stature, the impression he gave was that of a genuinely modest and somewhat humble man. He always seemed to look up to whomever he met on a casual basis. Albeit meeting Chang was always a welcome and delightful experience. Despite this self-effacement, Chang had a normal healthy ego and took justifiable pride in his own accomplishments.

One of Chang's most endearing attributes was his wonderful sense of humor. Before an audience his quick wit and facile repartee often had his audience in stitches. This was aided by an unusual feature in his manner of speaking, a rapid motion of the lower jaw. This made it appear that his often pithy and sometimes pungent quips were being ushered out with gnashing of teeth.

Chang was sensitive to any personal slight or any oversight of his scientific work. The aftermath of the discovery of the oral contraceptive was particularly nettlesome. As I have indicated elsewhere (*Journal of Andrology*, Nov.-Dec. 1992), "Chang sometimes figured in the series of clinical reports on these field trials but not to the extent that seemed deserving. Certainly there was no intent to downplay his role in this remarkable development and with the passage of time Chang came into his rightful dues as a co-founder of the Pill. It is much to Chang's credit that he never wa-

vered in his admiration and respect for his benefactor, Gregory Pincus."

In a final review summarizing his extensive studies, Chang noted rather pointedly and with some understatement that, "We have achieved a good deal towards the understanding of mammalian fertilization by simple biological experimentation." In this age of high technology and molecular probing, this may give encouragement to some who would aspire to extend the frontiers of knowledge by other means.

Chang was by any measure an extraordinary person. His mastery of the phenomenon of capacitation took six years of unrelenting effort. His demonstration of in vitro fertilization in a mammal took fourteen years. Such tenacity has become a rarity in biomedical research due to the tenuousness of financial support. Chang's rise to prominence as a Chinese scholar during a period of political unrest and social upheaval at home was also out of the ordinary by a wide, nay very wide, margin. Similarly, his contributions to human welfare are on a scale matched only by the few whose names are legend.

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