IN MEMORIAM: ROBERT L. KROC
A RELAXIN PIONEER, AND SO MUCH MORE

Robert L. Kroc had a long and distinguished career in the biomedical sciences, first as a researcher and later as president of a philanthropy. This tribute will focus primarily on Kroc's contributions in the relaxin field, not because his other work is any less important, but because it is the area in which the author has first-hand knowledge.

Most "relaxinologists" are familiar with the name Robert L. Kroc and many have had the good fortune to have enjoyed his company at the Endocrine Society Meetings and various international conferences and congresses on relaxin. While Bob Kroc is generally recognized as a disciple of Frederick L. Hisaw (*the Hallowed Father of Relaxin*), relatively few relaxin researchers know how much Bob, himself, contributed to the preservation and perpetuity of the relaxin movement during the difficult earlier years, not just by his publications, but by his actions in the research field, his judicious use of resources during his tenure as manager in a pharmaceutical company, and his insightful interactions with other investigators around the globe. We shall consider each of these aspects of his remarkable career.

Bob Kroc died on April 19, 2002, in Santa Barbara, CA, just two months before his 95th birthday. Born and raised in Chicago, Bob graduated from Oak Park and River Forest High School in 1925. From there he went to Oberlin College in Ohio, receiving a B.A. degree in 1929 and an M.A. in 1931. He then earned a Ph.D. in Zoology and Physiology from the University of Wisconsin in 1933. It was there that he met Alice Voelker, whom he married in 1934. Their marriage, which lasted 59 years. was blessed with two talented daughters, Alice Ann and Lois Sandra., six grandchildren and five great-grandchildren. There is no doubt that Bob's family contributed handsomely to his illustrious career. Wife Alice, who was herself trained in Zoology, provided intellectual stimulation and editorial skills, as well as moral support when the going got tough on "Mt. Olympus" (as Bob characterized the Warner-Lambert management headquarters in Morris Plains, NJ). Alice was also a gracious hostess, and she and Bob entertained scientists from all over the globe. Her imaginative entrees would have been envied by Martha Stewart (e.g., meatloaf stuffed with hot dogs or polish sausage!) Daughter Alice Ann's marriage to Hansjoerg Hattemer, a physician in Mainz, Germany, resulted in frequent trips to Europe by Bob and Alice, and gave the family a truly international flavor. Daughter Lois maintained a comfortable home for Bob after her mother's death in 1993, and was ever a thoughtful and caring companion on Bob's sojourns around the U.S.A. and to Europe and Australia.

From 1933-1944, Dr. Kroc was Instructor and then Assistant Professor of Zoology at Indiana University. In 1944 he began a career in biomedical research, joining the Maltine Company of Brooklyn, NY, which relocated to Morris Plains, NJ, and through various mergers, metamorphosed from Chilcott Laboratories into Warner-Chilcott and finally the Warner-Lambert Pharmaceutical Company. Bob had a remarkable record of achievement as Director of Physiology at the Warner Lambert Research Institute until he retired in 1969. During that time he directed research that led

to the development of Proloid® and Euthroid® for treatment of hypothyroidism, Simplastin® for measuring blood clotting time, and last but not least, Releasin® brand of relaxin for treatment of premature labor and threatened abortion.

The relaxin story is in itself a testimonial to Bob's intuition, attention to detail and courage to pursue the project according to his own best judgment; his approaches sometimes flying in the face of those of other Hisaw proteges (not to mention the Clinical Research and Marketing Departments at Chilcott and later, Warner-Lambert!)

Kroc's Ph. D. thesis had concerned adrenal cortical-ovarian relationships, so his only knowledge of relaxin came from Hisaw's Wisconsin lectures and publications. Thus, when Chilcott management chose to enter the relaxin field, Bob had to start the project literally from scratch; finding a reliable source of the hormone, developing an efficient extraction method, and setting up an accurate and reproducible bioassay procedure.

Bob first hired Swift and Company to collect swine ovaries. Whereas Hisaw and his students had painstakingly cut individual corpora lutea off the ovaries at the slaughterhouse, Bob realized that such a procedure was impractical for production scale-up. Instead, to increase the yield of relaxin in whole ovarian extracts, he trained the workers on the swine dis-assembly line to feel the uterus of each carcass as it passed and only collect ovaries from those that contained palpable fetuses. (Ovarian relaxin concentrations were known to be highest during pregnancy.)

The initial extraction procedure was adapted from that published by Hisaw's group, utilizing ammonium sulfate extraction of ground frozen ovaries. Much to Bob's surprise, the yields were 100-300 times those reported by Hisaw's team, causing him to question his conduct of the bioassay. The bonanza yields were, instead due to the large proportion of pregnant ovaries now being collected, thanks to his careful training of slaughterhouse personnel. Bob later made an additional important discovery that was to form the basis of a practical large-scale extraction procedure: unlike most ovarian proteins, relaxin was soluble in 70% acidifed acetone.

The only bioassay then available involved the subjective evaluation of the "relaxation" of the pubic symphysis of estrogen-primed guinea pigs by manual palpation of their pelvices. However, nowhere in the literature was there an adequate description of the method, let alone instructions on how to hold the guinea pigs! Working with his assistant, Shirley Rosegay, Bob adopted a procedure that always employed a control group treated with a relaxin reference standard preparation in each new experiment. Shirley developed a method for anchoring the squirming guinea pigs (ventral side up, firmly grasped between one's thighs!) so that the pubic symphysis could be palpated in a highly reproducible way. The positive response, a "relaxation" score of 4 or more on a scale of 0 to 6 was equated to the feel of pubic symphyses of guinea pigs in late pregnancy, where the consistency of the interpubic connective tissue is like that of foam-rubber. In addition, unknowns were coded, and the palpations performed independently and blindly, by two people trained in the procedure. Thus, a reproducible and statistically

reliable bioassay evolved, with respectable limits of error for such a highly subjective method.

The significance of the Kroc relaxin bioassay is underscored by the fact that many researchers (including Bob himself in the early going!), several of Hisaw's students, and investigators in two other pharmaceutical companies went totally astray by using a less rigorously controlled guinea pig bioassay. They wound up with virtually meaningless potency estimates that actually resulted in their discarding the major relaxin fractions from their ovarian extracts! These flawed guinea pig assays also led to publication of claims that serum relaxin levels increased throughout human pregnancy. Sensitive radioimmunoassays later showed the opposite: serum relaxin concentrations actually decreased with advancing human pregnancy, and were well below the limits of detectability of the bioassay. Again, thanks to Bob Kroc's carefully controlled bioassay, he never reported detection of relaxin in human pregnancy serum.

Reports then appeared that injections of relaxin induced separation of the pubic bones of estrogen primed, ovariectomized mice, as revealed by X-rays of their pelvices. Bob at once saw the advantages of adopting an objective bioassay instead of the subjective guinea pig method that had led so many investigators astray. However, the cost of the equipment and the labor-intensive procedure of anesthetizing mice for pre- and post-relaxin examination by X-ray were daunting. Then, while strolling with his wife Alice on a moonless night, he happened to hold a flashlight to the palm of his hand and was inspired to think "transilluminate!" This revelation led to the assembly of a binocular dissecting scope fitted with an ocular micrometer and a bevel-tipped lucite rod hooked to the light source: a simple but effective transilluminator.

I joined Chilcott Laboratories in 1954 and was assigned the development of an objective mouse interpubic ligament bioassay for relaxin. Armed with Bob's transil-luminating device that fitted neatly into the birth canal after simple dissection, Vivian Beach and I tested various combinations of estrogens and relaxin in immature intact, rather than ovariectomized, mice. Our main objective was to develop a method that would permit the use of at least 120 mice in a single assay, ruling out time-consuming procedures such as ovariectomy and daily dosing as had previously been used. A single dose of Upjohn's long-acting estradiol cypionate on day 1 was found to be as effective as 7 daily injections of estradiol-17β in priming mice for relaxin injection on day 7. A second problem, that purified porcine relaxin required use of a depot vehicle to be fully active in mice, was solved by Bob Kroc's contacts in the pharmacy department. They provided a hyaluronidase inhibitor, benzopurpurine-4B, which potentiated relaxin activity in mice some 300-fold compared to an aqueous solution.

Around this time, the Biochemistry Department, which had taken over the relaxin purification chores, was cranking out porcine relaxin samples faster than we could assay them. Although a uterine motility inhibition assay was used as a screen, Bob required that every important relaxin preparation be tested in both the guinea pig and mouse pubic symphysis bioassays, each with 120 animals equally divided between three dose levels of a standard and unknown. To assure quality control, he convinced the company to set

aside a large batch of porcine relaxin extract to use as a reference standard. He further persuaded management to allow him to send a portion of this relaxin standard to the NIH for distribution to qualified investigators, and also to provide relaxin extracts of similar potency to other researchers in the U.S. and Europe. In addition to providing universal standardization of relaxin activity, this foresight paid handsome dividends, in terms of feedback on research activities of other laboratories; we were privy to discoveries from around the world long before they appeared in print.

As a final thought regarding the basic research on relaxin, it should be mentioned that our little team (Bob, Vivian Beach and I) working closely together, published several key papers that delineated the precise roles of estrogen, progesterone and relaxin in uterine growth, cervical dilatation and parturition in rats and mice.

As soon as the biochemists produced a partially pure (about 5% relaxin/95% other pig peptides!) but nontoxic injectable relaxin preparation, clinical trials were launched, the initial target being arrest of premature labor and threatened abortion. As luck would have it, the movie star, Carole Landis (the Dolly Parton of that generation) was one of the early cases of premature labor whose fetus was "rescued" by Releasin® brand of relaxin. It was, perhaps, predictable that the company's desire to have a product, coupled with the unfounded exuberance of obstetricians in both the Clinical Department and the field led to instant claims of success of Releasin® in its early trials, and Marketing launched a sales campaign. Bob Kroc bucked the clinicians and management by insisting that further studies be controlled with an inactive placebo and conducted double-blind. Although many of the clinical investigators claimed that Releasin® was so active they could immediately tell which patients received it, when the dust cleared and the samples were decoded, there were no significant differences between Releasin® and placebo!

Other clinical trials were launched to use Releasin® for dilatation of the uterine cervix. Ignoring Bob Koc's pleas for human pharmacology in non-pregnant women to confirm animal experiments which clearly showed relaxin was a powerful dilator of the cervix, the drug was, instead, injected into women in labor. Predictably, there were no differences between Releasin- and placebo-treated women, because these women already had their own relaxin in circulation.

Lastly, there were promising trials of Releasin for treatment of peripheral vascular disease and scleroderma, conducted at the Miami Heart Institute by Robert Boucek and Gus Casten, who willingly controlled their studies with a placebo injection. At last! A ray of sunlight for Bob and his team! But again, it was not to be. The biochemists had now perfected the extraction methods to provide essentially pure relaxin: a 20-fold increase in purity over previous preparations! Bob Kroc and those of us on his team celebrated this breakthrough, but our joy was short-lived: the FDA stepped in and asserted that the highly purified preparation was so different from the original, that a New Drug Application would have to be filed, complete with safety studies and new clinical trials! That did it for management, and the project was summarily dropped!

I then moved to CIBA Pharmaceutical Company, where with hope I carried the relaxin torch in Bob's tradition, and armed with his advice to be guided by one's own instincts and not to be dissuaded from one's goals by the wishful thinking of management. "The prisoners run the prison", he would say, and I have never forgotten. It should also be mentioned that, recently, several other pharmaceutical companies and biotech firms have attempted to develop relaxin for clinical use; sadly, repeating many of the mistakes of the past. Unfortunately, there was no Bob Kroc available to lend his remarkable instincts, enthusiasm and attention to detail to these endeavors.

Although this tribute has been concerned primarily with relaxin, it should be emphasized that Bob Kroc was far from uni-dimensional in his research interests. In 1969, Bob and Alice moved to Santa Ynez, California where he became President of the Kroc Foundation, a philanthropic foundation initiated by his brother, McDonald's Corporation founder, Ray Kroc. The Kroc Foundation dedicated itself to furthering scientific research in the fields of diabetes, multiple sclerosis, and arthritis through sponsorship of conferences at the Foundation headquarters at the J & R Ranch and through the giving of research grants. Conferences promoted scientific discourse on an international scale in these and various other scientific fields, many conferences resulting in publications, including a memorable conference on relaxin in 1980. Although disbanded in 1985, the Foundation, thanks to Bob's leadership and foresight, continues to impact ongoing scientific programs through its endowed lectureships and professorships. Over 1600 research grants were awarded to U.S. institutions and 28 to institutions in eight foreign countries.

Bob Kroc was a member of a number of scientific organizations, including the Endocrine Society and the American Physiological Society. He was president of the American Thyroid Association in 1971-72, a Fellow of the New York Academy of Sciences and a board member of the American Diabetes Association, National Multiple Sclerosis Society, Santa Barbara Chapter of the Arthritis Foundation, Hauptman-Woodward Medical Research Institute of Buffalo NY, and the Sansum Medical Research Institute of Santa Barbara CA.

Bob Kroc received an honorary Doctorate of Science from Oberlin College in 1979, presented with the inscription, "Devoted alumnus, innovative and inspiring biologist whose skills and humanitarian concerns have made the healing arts more effective everywhere". These humanitarian concerns were, perhaps best exemplified by his seeking and obtaining institutional support for several overqualified technicians to enable them to pursue advanced degrees at medical or graduate schools, and by the unsolicited tributes of his adoring grandchildren on his passing.