From chemical warfare to breast cancer management

Elwood V Jensen

In reviewing the circumstances that led to the research that is being recognized by the Lasker Foundation, I am struck by the number of factors, many of them fortuitous, that played important roles in the process. These are as diverse as World War II, the Swiss Alps, sympathetic mentors (such as Morris Kharasch and Charles Huggins), faulty vision and the location and structure of the University of Chicago, where these investigations were carried out.

After graduating in 1940 from Wittenberg College, in Springfield, Ohio, with a major in chemistry, I had little doubt that I would pursue an academic career in organic chemistry. By the autumn of 1941, I had completed the qualifying examinations in chemistry at the University of Chicago and begun graduate research under the supervision of Morris Kharasch. But then came Pearl Harbor and the ensuing war, which altered all plans. Although I had a private pilot's license and wanted to join the air corps, my vision did not meet their standards, so I settled for research on chemical warfare in a group that Kharasch had organized in the Department of Chemistry (**Fig. 1**). Despite two stays in the hospital, when novel reactions of toxic substances proceeded more vigorously than anticipated, this research experience stimulated my interest in biochemistry and physiology. As a result, I decided that when the opportunity arose, I would apply my expertise in chemistry to biomedical studies.

After the phasing-out of the poison gas work after the fall of Germany, I spent a year on a synthetic-rubber project, where I made two important discoveries[1,2], mostly by accident, that greatly enhanced my standing with Professor Kharasch. Thus, when I indicated my desire to learn about steroid hormones, Kharasch used his considerable influence to help me obtain both a place in the laboratory of Leopold Ruzicka at the Swiss Federal Technical Institute (ETH) in Zürich and a Guggenheim fellowship to support the study. So, in the autumn of 1946, my wife, Mary, and I departed for a year in Switzerland.

The Swiss experience

Not only did the laboratory research in Zürich set the stage for later work in the field of steroids, but the stay in Switzerland also involved two nonacademic experiences that influenced my subsequent career. Through friends at the laboratory, I was introduced to a young man who had just developed a single-pan, constant-sensitivity analytical balance. Erhard Mettler wanted to market his balance in the United States, but no one whom he contacted had any interest in an instrument with only one pan, because everyone knew that an analytical balance had two. After riding on the back of his motorcycle (he could not afford an automobile) to see his small factory in a Zürich suburb, I agreed to help introduce this novel instrument to the United States. Mary formed a small company that imported and sold the first six balances, whereupon the Fisher Scientific Company appeared on the scene and agreed to take over, leaving me free to concentrate on cancer research with a modest but important supplement to a young assistant professor's salary. This was my first introduction to the

Figure 1 National Defense Research Council project on chemical warfare at the Department of Chemistry, University of Chicago, 1944. Elwood Jensen is third from right.

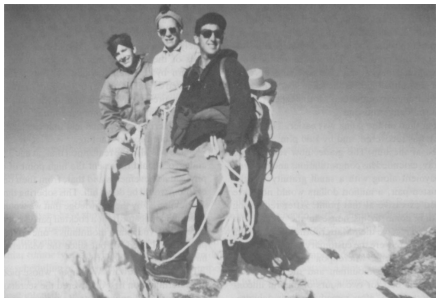

Figure 2 Summit of the Matterhorn, August 18, 1947. Left to right: Kyle Packer, Elwood Jensen and Swiss guide Gustav Julen. From ref. 3.

reality that something unconventional may not be accepted immediately, even though it is much better than what is currently available.

My second noncurricular lesson involved the Swiss Alps. Like most foreign visitors to Switzerland, I could hardly wait to see the Engadin and especially the famous Matterhorn. In this instance, seeing was not enough, and, even though I had no previous experience with mountaineering, my great enchantment led me to accept an invitation from a fellow student who was an experienced climber to join him and his Swiss guide in undertaking the ascent. As described in more detail elsewhere[3], the project was successful, and the view from the top was spectacular (**Fig. 2**), even though, as a novice, I found it the hardest physical challenge I would ever encounter. But a valuable lesson came later, when my curiosity about the reason the Matterhorn was the last major peak in Europe to be climbed led me to delve into its history. Before the first successful ascent by an Englishman named Edward Whymper, most attempts to reach the summit had been made from the Italian side, which appears to be the most favorable, whereas the northeast face that is usually seen in photographs appears to be a sheer wall of rock. Whymper and his party decided to try this 'impossible' approach and became the first to conquer the Matterhorn, even though four of the party of seven died in an accident on the descent[3]. For me, though, the lesson of the alternative approach lived on to have an important influence in the discovery of the estrogen receptor.

Move to the medical faculty

Near the end of our stay in Zürich, I received a message from Kharasch at the University of Chicago advising me not to accept any position until I returned, because there was someone he wanted me to meet. This person was Charles Huggins of the Department of Surgery, who was planning to establish an interdisciplinary unit for cancer research within the medical school. Kharasch had recommended me to Huggins as an organic chemist interested in physiological problems, thinking (correctly) that this was the kind of position that I would find attractive. After meeting with Huggins, I had no doubt that this was the place for me. In 1947, as an organic chemist, I became an assistant professor of surgery until the Ben May Laboratory for Cancer Research was officially established as an independent entity in 1951, initially consisting of Charles Huggins (**Fig. 3**), Albert Lehninger, Paul Talalay and me.

It was Huggins who had the most important influence on my career. He taught me medicine, and I taught him some chemistry. Although he was a urologist and would win the Nobel Prize in 1966 for his pioneering work on the antiandrogenic treatment of prostatic cancer, he was also interested in estrogens and breast cancer. When the advent of cortisol replacement made adrenalectomy feasible, he introduced this treatment for advanced breast cancer in postmenopausal women[4]. In the early 1950s, I was fascinated when he showed me how minute amounts of estradiol administered to immature rats can cause spectacular growth of the uterus and other reproductive organs, and I resolved to find out just how it did it.

Discovery of the estrogen receptor

During the 1950s, when enzymes were the major focus of biochemistry, many investigators were studying the mechanism of estrogen-stimulated growth by attempting to identify the enzyme system that first shows enhancement after exposure of the target tissue to hormone. Several pathways were found to show early stimulation. With many possibilities to choose from, and the hormone itself the only thing known with certainty to participate in the earliest stage of estrogen action, it seemed that one might obtain valuable clues by taking an alternative approach. Rather than asking what the hormone does to the tissue, one could find out what the tissue does with the hormone. In the case of estrogens, such studies were beset by the complication that these hormones are active in such tiny amounts. Thus, to detect and study the hormone that moves to the target tissues, one would need labeled steroid of much greater radioactivity than had been known until then. Herbert Jacobson, a postdoc who had just finished his doctoral studies in chemistry with Morris Kharasch, and I designed a microhydrogenation apparatus in which the double bond in 3-mg quantities of 6-dehydroestradiol could be reduced catalytically with pure tritium gas to yield 6,7-tritiated estradiol of specific activity sufficient to detect one-trillionth of a gram[5].

After devising an improved method for counting tritium in animal tissues, we administered a physiological dose (90 ng)

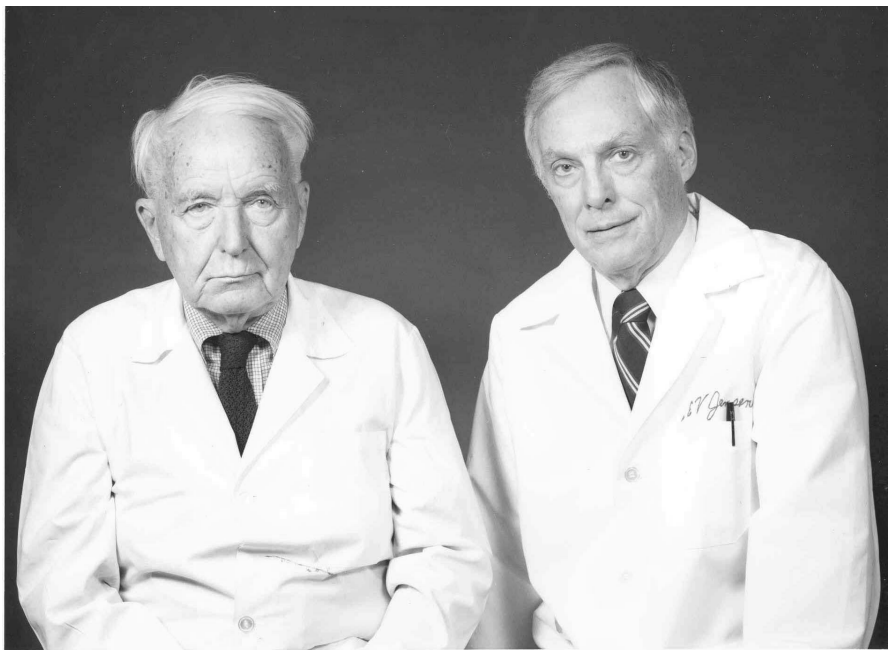

Figure 3 Charles Huggins and Elwood Jensen, Ben May Laboratory for Cancer Research, University of Chicago, about 1975.

of our tritiated estradiol to immature rats and determined the amount and nature of the radioactivity in the blood and in different tissues at various time intervals[5]. As shown in **Figure 4**, the radioactivity in the nontarget tissues reflects that of the blood, whereas the reproductive tissues show a much higher uptake and retention for a prolonged period. After a few minutes, the blood contains a mixture of tritiated metabolites (apparently produced in liver and kidney), from which the uterus and vagina take up and retain only estradiol with a concentration of more than 100 times that in blood after 2 hours. Thus, the tissues sensitive to stimulation by estrogens seemed to contain a specific component that binds estradiol without changing it chemically.

At the time these studies were undertaken, the accepted mechanism for estradiol action was that its 17-hydroxyl group was enzymatically oxidized by one coenzyme and reduced again by another, thereby producing NADPH at the expense of NADH ('transhydrogenation'). Our finding of apparently unchanged estradiol bound in the target tissues did not invalidate this hypothesis, because it was possible that the reversible oxidation-reduction process did take place but the equilibrium was tipped so far toward reduction that no tritiated estrone could be detected. To address this question, we synthesized 17α-tritiated estradiol, which, if oxidized, would lose its tritium. Then we devised a simple procedure for quantifying the 6,7- and 17-tritiated steroids in the presence of each other, administered an equal mixture

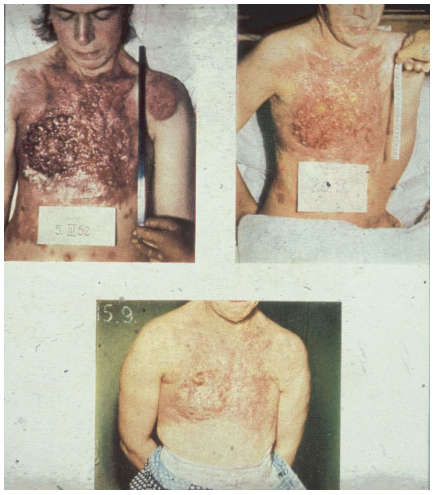

Figure 5 Patient with metastatic breast cancer 3, 8 and 18 months after hypophysectomy. From ref. 15.

of the two isoforms to immature rats, isolated the tritiated estradiol from the uteri and showed that the ratio of the two was the same as the ratio in the mixture injected[6]. These results caused the demise of the transhydrogenation hypothesis and convinced all but the most diehard enzymologists that estradiol binds to a characteristic component of target cells to exert its physiological effect without itself being chemically altered. That this binding protein is a true receptor, actually involved in the uterotropic process, was established by our subsequent observation that the progressive inhibition of estradiol uptake in the rat uterus by increasing amounts the antiestrogen nafoxidine closely parallels its inhibition of uterine growth[7].

A two-step mechanism for estrogen action

After our report in 1962 of the nonmetabolic nature of estradiol action[6], representatives of the New England Nuclear Company paid a visit to our laboratory to learn how we synthesized our tritiated estradiol and, by 1963, this and other tritiated hormones became commercially available so that other laboratories could undertake hormone-tracking experiments. A detailed description of the studies of many investigators, leading to our present understanding of the molecular biology of steroid hormone action, is beyond the scope of this commentary. I will only mention Toft and Gorski's introduction of the use of sucrose-density sedimentation[8] to detect the complex of tritiated estrogen with the native receptor protein found in the cytosol fraction of untreated uterine homogenates. This technique allowed us to show that the receptor protein that becomes tightly bound in the nucleus after administration of hormone is different from the native form of the protein[9]. Along with other observations[10,11], this led to the concept that the basic function of the hormone is to free the native receptor from its associated proteins, converting it into a biochemically functional form[12] that can bind as a dimer to the target gene and serve as a transcription factor. This two-step process was later shown by many groups to be a common property of the action of all classes of steroid hormones.

Antibodies to estrogen receptor

In studies before 1977, the receptor was usually recognized by its ability to bind labeled hormone. Several laboratories attempted without success to prepare

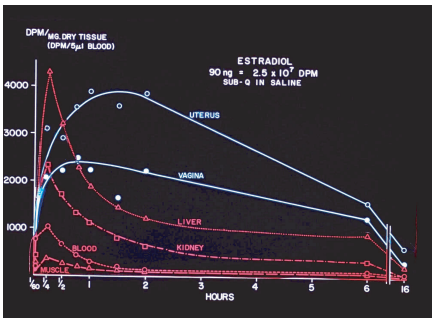

Figure 4 Selective uptake and retention (without chemical change) of tritiated estradiol by reproductive tissues of the immature rat. DPM, disintegrations per minute. From ref. 5.

specific antibodies to estrogen and other steroid hormone receptors, leading to speculation that perhaps such receptors are so ubiquitous that they are not immunogenic. We considered the possibility that antibodies to the estrogen receptor form soluble immune complexes and thus may not be detected by conventional immunoprecipitation techniques. So we undertook an alternative approach by using sucrose-gradient sedimentation to identify suspected antibodies by their ability to shift the sedimentation peak of the receptor labeled with tritiated hormone as a marker. This technique worked beautifully, allowing us to obtain the first polyclonal[13] and monoclonal[14] antibodies to any steroid hormone receptor. These antibodies provided valuable reagents for basic studies and for the immunoassay of the receptor in tissue and tumor specimens, and other researchers used them for the original cloning of the estrogen receptor.

Selection of therapy for breast cancer

It has long been known that some human breast cancers retain the estrogen dependency of their tissue of origin. Removal of supporting hormone by excision of the ovaries in premenopausal patients with advanced disease, or of the adrenals[4] or pituitary[15] gland in postmenopausal women, results in striking remissions in about one-third of cases. More recently, similar remissions have been obtained by blocking estrogen biosynthesis with aromatase inhibitors or hormone action at the target level by using tamoxifen or other antiestrogens. The remissions obtained by endocrine manipulation (**Fig. 5**) are superior to those seen with chemotherapy, yet because only one out of three patients responds, there was a need for a means to predict hormone dependency in advance, so that most patients would not be first

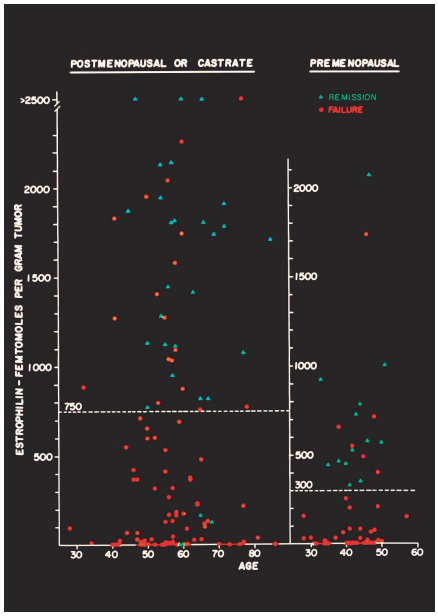

Figure 6 Correlation of estrogen receptor content with response of breast cancer to endocrine ablation in 160 patients with metastatic disease. Green indicates objective remission, and red indicates failure. Receptor content was determined by binding of tritiated estradiol. Age refers to patient's age in years. From ref. 17.

placed on an ineffective therapy delaying the trial of alternative treatment, such as chemotherapy.

The recognition that estrogenic hormones exert their action in combination with specific receptor proteins suggested that, whereas hormone-dependent breast cancers probably contain estrogen receptors for the hormone to act, those that have escaped from hormone dependency may have lost these receptors. Early testing of this hypothesis with patients undergoing adrenalectomy[16] showed that mammary tumors with low or negligible receptor content rarely respond to such treatment, whereas most (but not all) patients with numerous receptors obtain benefit. These results have been confirmed by many subsequent studies[17] (**Fig. 6**), and estrogen receptor analysis of either primary or metastatic cancer specimens, preferably by immunoassay, has now become standard clinical practice.

ACKNOWLEDGMENTS

And so a road that began with the search for more effective agents to eradicate fellow human beings slowly meandered its way to a program for reducing or eliminating human suffering. During this journey, the author has received assistance, enlightenment, support and encouragement from many sources and individuals. I am grateful to the gifted students, fellows, colleagues and, by no means least, technical assistants who are the backbone of continued research effort. I thank my late wife, Mary, who, with patience and fortitude, brightened the earlier part of the journey, as well as my present wife, Peggy, who will be there on October 1 to celebrate the occasion. And I am thrilled to participate in the 2004 Basic Medical Research Award of the Albert Lasker Foundation; may this organization continue its most valuable mission in stimulating medical research.

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