Classic Studies of Mammary Development and Milk Secretion: 1945 – 1980

Margaret C. Neville

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Abstract On June 20, 1947 a meeting at the Royal Society of Medicine in London was entitled “Discussion on Some Recent Developments in Knowledge of the Physiology of the Breast” [1]. The major questions outlined by the speakers were: how does the structure of the breast change with reproductive stage? what is the role of the basal cell layer in the mammary epithelium? how is milk composition related to diet? and what is the basic physiology of milk secretion including its hormonal regulation? All these questions were attacked vigorously in laboratories mostly in England and the United States, but researchers in France, Germany, and Austria also weighed in. Our purpose in this edition of the Journal is to show, through the presentation of seminal papers and key references, how the research of that period, prior to the molecular revolution of the last three decades, laid the groundwork for our current understanding of the morphology, developmental biology, and physiology of the functional mammary gland. This knowledge provides the groundwork for our current research into the molecular mechanisms involved in milk secretion and its regulation.

Milk Composition In 1926 Margaret Lowenfeld and her colleagues at the London School of Medicine for Women wrote: “A VERY extensive literature has grown up during the past half century, on the subject of the chemistry of normal human milk. So vast has this literature become, that the task of absorbing and correlating its conclusions into a coherent understanding of the subject as a whole has become one of extreme

difficulty and laboriousness” [2]. And this article refers only to human milk! The proteins of milk, being abundantly available, have been the object of research by protein chemists for more than 150 years. An 1908 article in the *Journal of Biological Chemistry* by G.A. Olsen contains references to publications going back to 1854. By the late forties the composition of bovine, goat, and human milk was well established, and Robert Jenness at the University of Minnesota began a serious study of the composition of milks of many species [3, 4]. Jenness, who founded the Gordon Conference on Milk (now the Gordon Conference on Mammary Gland Biology) in 1971 with Stuart Patton, was known to be a consummate teacher in the Biochemistry Department at the University of Minnesota. As a graduate student with Professor Samuel Coulter during World War II he was in part responsible for the development of a dry whole milk that could be transported to the troops and reconstituted without a significant loss of quality. We were unable to include any of Jenness’s extensive publications in this volume; fortunately a comprehensive review published by the *Journal of Dairy Science* in 1986 is available on line without cost [5]. This article, which also discussed many nutritional aspects of lactation, is well worth reading.

Morphological Studies The developmental morphology of the murine mammary gland was published in 1934 as a series of very beautiful camera lucida drawings of the gland as the mouse proceeded through the reproductive stages from the virgin, through pregnancy and lactation, to involution [6]. By the late 40’s advances in light microscopy techniques were such that K.C. Richardson was able to depict the structure of the myoepithelial cells responsible for milk let-down in the lactating gland [7] — that paper is reprinted here.

M. C. Neville (✉)
CU Denver School of Medicine,
Aurora, CO 80045, USA
e-mail: Peggy.neville@ucdenver.edu

The electron microscope, invented in 1931 by Max Knoll and Ernst Ruska at the Technical College in Berlin, became widely available to biologists in the 1950's. Mather and Keenan stated in a 1998 review [8], "The stunningly beautiful organization of mammary cells during lactation was evident from the earliest electron micrographs". Researchers throughout the world began to produce dazzling images of the mammary epithelium in all stages of development [9–14]. Most of these articles are too long to reprint, but Bargmann and Welsch summarized their findings at a Symposium held in 1968 at the School of Veterinary Medicine of the University of Pennsylvania, producing for us a drawing that shows all the intracellular organelles with which later researchers would be concerned. The book that contains this article and many other articles of great historical interest, *Lactogenesis: The Initiation of Milk Secretion at Parturition* (Philadelphia, University of Pennsylvania Press, 1969) was, at this writing, still available in the antique book market and is well worth examination. By 1973, Michelle Ollivier-Bousquet at Jouey-en-Josas, France had perfected immune-electron microscopy of the lactating rat gland, allowing her to trace proteins in their secretory path through the alveolar cell [15]. Peter Wooding at Babraham had produced superior images showing the secretion of the milk fat globule [14]. One of the finest electron microscopists of the time, Dorothy Pitelka, was head of the electron microscope laboratory in the Department of Zoology at Berkeley, and began to publish astonishing images beginning in 1960 [12, 16]. Perhaps her best work, reprinted in this issue, is a study of cell contacts in the developing and lactating mammary gland [17].

The Physiology of Milk Synthesis and Secretion. The insight into the cellular structure of the mammary secretory cell gained from examination of electron micrographs had a huge influence on lactation physiologists. Until this time, physiologists were accustomed to viewing the cell as a bag of salts and soluble proteins whose composition was determined largely by nutrient and ionic fluxes across a limiting membrane. Views of the mammary cell, like the drawing depicted in Fig. 1, required a whole new approach to physiological processes. Jim Linzell, a veterinarian who had started his research career at the University of Edinburgh moved in 1950 to the new Institute of Animal Physiology, Babraham, Cambridge, UK. Here he began a prodigious series of studies on the physiology of milk secretion in the lactating goat. By 1970 he had been joined by Malcolm Peaker, whose background was in the biology of the avian salt gland. In 1971 they published a comprehensive treatise that provided enormous insights into the relation between the structure of the mammary alveolar cell and its secretory activity [18]. They pointed

Figure 1 Diagram of the milk secreting cell as interpreted from electron micrographs [18]. Used by permission of Physiological Reviews.

out that the mammary epithelium secretes more than three times its volume daily [8] of a fluid containing a diversity of constituents synthesized and secreted by several cellular pathways. Proteins were synthesized in the endoplasmic reticulum and transported to the Golgi, where they underwent substantial modification before being secreted in a uniquely expanded secretory vesicle that caused some controversy in early days. These vesicles are quite clear in Fig. 1, where they are shown as clear vesicles containing electron opaque particles that had been found earlier [19] to represent casein micelles. The expansion of the lumen in these secretory vesicles was shown a bit later to be due to the osmotic activity of the lactose, synthesized in this compartment, which draws water and ions into the vesicle. The definitive paper by Kuhn and White detailing the evidence for lactose synthesis in the Golgi compartment [20] is reprinted in this issue.

Lipid, represented by the black spheres in the figure, was found to be secreted by a unique pathway: triglycerides synthesized in the cytoplasm move toward the apex of the cell where they fuse to form larger lipid droplets that in turn fuse with the apical membrane and are budded into the lumen [14]. Often these droplets carry a crescent of

cytoplasm with them, suggesting to Linzell and Peaker that secretion of the milk fat globule utilizes a process akin to apocrine secretion [18]. This opinion was not shared by the consummate electron microscopy expert Wolfgang Bargmann at the University of Kiel, who considered that apocrine secretion was an old concept that should be discarded [21].

By the 1970's a great deal was known about the biochemistry of the synthesis of milk triglycerides. Isotope studies carried out at the National Institute for Research in Dairying at Shinfield in the lactating goat showed that 40% of fatty acids in milk fat were synthesized *de novo* from acetate and β -hydroxybutyrate; the remaining 60% were taken up as pre-formed fatty acids from the plasma [22]. The radioactive goats resulting from these experiments were famous in NIRD folklore. In non-ruminant species glucose replaced acetate as the substrate for *de novo* lipid synthesis. Lipids synthesized in the mammary alveolar cell have the unique characteristic of being short or medium chain in length, that is, containing up to 16 carbons while 18 or more carbons are characteristic of fats synthesized in other mammalian organs as well as plants [23]. Early termination of fatty acid chain length was shown by Stuart Smith to be the result of a special mammary enzyme associated with fatty acid synthase [23, 24]; a publication reviewing the laborious work necessary to identify this protein is reprinted here. Also reprinted here is an article showing how enzymes involved in the process of *de novo* lipogenesis change to the onset of lactation in cows [25].

Not all the lipids in milk are synthesized in the mammary gland. Several sources of pre-formed fatty acids are available from the plasma: VLDL particles synthesized by the liver or chylomicra bearing triglycerides absorbed from the diet interact with lipoprotein lipase, present on capillary walls, which hydrolyzes the triglycerides into fatty acids and glycerol; these are then taken up into the alveolar cell for incorporation into the cytoplasmic lipid droplet [26], a process which appeared to take place in the endoplasmic reticulum [27].

Alveolar Differentiation and Secretory Activation In his 1947 summary [1], Folley defined “lactogenesis” as the onset of lactation and “galactopoiesis” as the production of milk, terms that have been widely used in the dairy science and human lactation literature but have now been supplanted in the general lactation literature by “secretory activation” and “lactation”, respectively [28]. Hartmann and others noted that the initiation of lactation took place in two stages [29]. The first stage occurred during late pregnancy when variable amounts of lactose could be observed in the mammary gland of the cow; he called this stage “lactogenesis I” [30]. We now call it secretory differentiation. In rabbits medium chain triglyceride synthesis began

in mid-pregnancy [31]. Secretory activation is defined as the onset of copious milk secretion. It is marked by a substantial increase in lactose synthesis [32, 33], by changes in expression of genes involved in milk protein and lipid synthesis [34], and by a change in the permeability of the mammary epithelium [17, 35]. In pregnancy the mammary epithelium is extremely leaky such that proteins such as lactalbumin can pass from the lumen to the blood stream and blood proteins can enter the milk space [36]. During lactation the epithelium is among the tightest known, actually maintaining a potential difference up to -35 mV [30]. The switch between the two states was studied simultaneously in the early 1970's by Linzell and Peaker at Babraham and Dorothy Pitelka at Berkeley. The papers resulting from what was really a *tour de force* in each laboratory are reprinted here [17, 37].

Hormonal Control of Mammary Development and Milk Secretion

The hormones that regulate mammary development in the virgin were known in the 1930's from experiments like those of Turner and Frank at the University of Missouri [38]. They found that an estrus-producing factor isolated from cow urine produced the full extent of ductal development in rats or in castrated male rabbits. Adding an extract of the corpus luteum could produce the full extent of alveolar growth characteristic of pregnancy. It did not take long for the active principles to be purified as estrogen and progesterone, and by 1947 the role of a pituitary factor was also evident [39]. Several relevant pituitary factors were at hand: Prolactin had been discovered in 1928 and the purified hormone was available by the 1940's [40]. Growth hormone was found in many studies to enhance the lactogenic effects of prolactin [41, 42], an observation that eventually led to the utilization of bovine somatotrophin for augmentation of lactation in dairy cattle. ACTH in its role of stimulating glucocorticoid and TSH in its role of stimulating thyroid hormone were also possibilities [43].

This then was the state of affairs when extensive endocrine ablation and replacement studies were begun in a number of localities. In the Department of Anatomy at Berkeley William R Lyons, working with the famous endocrinologist Herbert M. Evans, began to take a serious interest in the regulation of mammary development and function, moving to the Medical School of the University of California in San Francisco about 1958, where he continued this work. S.J. Folley was the leading researcher at NIRD, Shinfield, England from 1932 to his death in 1970. He recruited many scientists including Isabel Forsyth and Alfred Cowie to examine aspects of lactation ranging from the biochemistry of lipid synthesis to the hormonal regulation of mammary development and lactation. In the States, scientists at NIH under the leadership of Yale

Topper [44, 45] were actively studying the endocrine control of mammary development. We reprint one article that exemplifies the approach used in the 1950's, an article on the hormonal control of mammary development and lactation in rats that is co-authored by a researcher from the NIRD, Shinfield, Alfred T. Cowie, and one from Berkeley, William R. Lyons [46]. It should be noted that the endocrine ablation studies utilized by Cowie and Lyons missed one important hormone, insulin. Because ablation of the pancreas produced so many metabolic defects, a direct role of insulin on mammary development could not be inferred from ablation replacement experiments such as these. In fact, the role of insulin in the regulation of lactation is only recently beginning to be understood [47, 48]. The *in vitro* approach pioneered with mammary explants in the laboratories of S.J. Folley [49] and Yale Topper provided solid evidence that insulin — or an insulin like substance such as IGF1 or IGF2 — was also critical to the regulation of mammary development [50].

At the conclusion of all of these experiments there were still significant questions about the hormonal mechanism that triggers secretory activation. This problem was solved by a young biochemist from Birmingham, Nicholas J. Kuhn, who showed from the appearance of lactose in the mammary gland of the late pregnant rat that the fall in progesterone associated with parturition was the trigger for secretory activation in rats, and that changes in glucocorticoid, estrogen, and prolactin do not constitute the critical signal. The article which describes these elegant experiments [33] is also reprinted here.

The final paper on hormonal regulation came from the laboratory of a young Jeffrey Rosen, who had recently moved from Roswell Park in Buffalo NY to Baylor College of Medicine at the invitation of Bert O'Malley. This ground breaking publication on the regulation of casein message during mammary development [51], looks forward to the next 3 decades of mammary gland biology during which the molecular principles governing the regulation of all the synthetic reactions that go into milk synthesis were to be established. The molecular era has many seminal papers, including more by Rosen, that have vastly increased our knowledge of the biology of the normal mammary gland and breast cancer, but that is another topic for another time.

Transplantation into the Mammary Fat Pad From the time of its establishment in the early 1950's, the Cancer Research Genetics Laboratory at Berkeley was focused on animal models for the study of breast cancer. Kenneth DeOme, the leader of the laboratory, realized that, in an era long before the advent of planned genetic mutations, the ability to transplant precancerous lesions and even tumors from one animal to another was crucial to the study of carcinogenesis. The story of his development of the cleared

mammary fat pad model is told in the introduction to the paper by Charles Daniel and colleagues, reprinted here [52]. Daniel undertook serial transplantations of pieces of the normal mammary gland and precancerous lesions from one mouse to the cleared fat pad of another, providing clear evidence that the gland contains progenitor cells that are able to reconstitute an entire mammary gland. These experiments form the basis for the extensive current research on mammary stem cells [53].

An Apology Many wrenching decisions had to be made in the choice of papers reprinted here. We picked papers on work carried out prior to 1980 that made significant advances in our understanding of the biology of the normal mammary gland, that were representative of the work of a particular laboratory, that were short enough so that we didn't exceed our page budget, and for which the copyright holders would provide permission to reprint at a reasonable fee. Even with these limitations many, many seminal papers were passed over. We have tried to quote some of these in our introductions to the reprinted papers, but have missed many. The brief introductions to each paper are meant to reveal the scientific atmosphere in which the work was done, to give a flavor of the authors involved where possible, and to show how this work is informing our current understanding of the biology of the mammary gland.

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