NOTE TO USERS

TITLE

STUDIES ON LUTEINIZATION IN THE

OVARY OF THE ALBINO RAT.

BY

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Studies on Luteinization in the Ovary of the Albino Rat

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INTRODUCTION

The earliest description of luteinization, a metamorphosis consisting essentially of the intracellular accumulation of lipoid inclusions, in the mammalian ovary is usually attributed to the monograph of von Baer, "De ovi mammalium et hominis genesi". In the century or more which has elapsed since that early account, numerous investigators have placed their evidence on the balance which will one day come to rest at an obvious truth. In the meantime considerable controversy exists concerning the precise origin of lutein tissue in the mammalian ovary. Today, two schools of thought are represented in the literature. The older or monistic group maintaining that lutein tissue is derived solely from the granulosa cells of the ruptured follicle, stands opposed to the somewhat more recent and dualistic view which maintains that both the theca interna and the granulosa elements play a part in the formation of the parenchyma of the lutein body. At the present time, the most readily acceptable view in reference to the higher mammalian forms derives the corpus luteum mainly from the granulosa permitting the theca interna to add a discontinuous layer of theca-lutein cells about the periphery of the mass. In the case of the smaller laboratory animals, most of which have been studied rather carefully, the corpus luteum is seen to consist of a single parenchymatous cell type. These cells are

derived, according to the majority of recent investigators, entirely from the granulosa layer. With the advent of recent work in endocrinology, the importance of the theca interna has been sharply outlined, and it would seem of considerable interest to investigate these curious and little known cells in greater detail. Do they play only a brief role in the complex ovarian endocrine system, or do they perhaps luteinize?

In seeking an answer to this question, it was learned that the rat had never been adequately studied in this respect. Furthermore, because of his convenient size and the ease with which hypophysectomy may be performed, the rat has been and no doubt will continue to be extensively used for endocrine research. It would then seem wise to establish with as great certainty as possible the cellular relationships of the rat ovary. This paper represents studies on luteinization in the ovary of the albino rat. The material classifies itself into two main subdivisions which cannot be considered simultaneously. The first subdivision consists of a series of luteinizing follicles, young corpora lutea, which were obtained under conditions conforming as closely as possible to the normal. The second subdivision consists of observations made on a group of structures representing atypical luteinization patterns, which may be classified as 'accessory corpora lutea'. The conclusions to be drawn from a study of the two series of specimens are quite similar and will be discussed together.

HISTORICAL BACKGROUND

Although the corpus luteum was first described in the sixth century by Volcherus Coiter it was not until much later that the first studies on the development or histogenesis of the lutein body were made. The earliest of these latter studies is thought to be that of von Baer (1827), who described the development of corpora lutea in the ovaries of the cat and swine. Considerable controversy exists over von Baer's original interpretation and description. When one considers that his paper was written before the introduction of the microtome, good fixatives and biological stains as we know them today; it is not surprising that the early descriptions were not entirely clear. It nevertheless seem apparent that he recognized the several layers of the follicle and distinguished sharply between the membrana granulosa and the two-layered thecal sheath. He further defined the inner layer of the theca as being cell-rich; while the outer layer was more fibrous in nature. According to von Baer, it was this inner cellular layer of the theca which, after rupture of the follicle, was solely responsible for the formation of the corpus luteum. Corner (1919) does not agree that the above is the precise interpretation of von Baer's original paper. However, the majority of von Baer's contemporaries, in general, are in accord with the presented view.

It is interesting to recall here that von Baer's

work preceded by ten years the introduction of the Cell Theory by Schleiden and Schwann. The work was done largely with the aid of a microscope, free-hand sections, and teased preparations. A few years later Patterson (1840) published a rather careful account of a macroscopic study of a series of corpora lutea. His material consisted of a group of ovaries taken from man, the ewe, sow, dog, cat and the rabbit. Without the aid of a microscope he was forced to conclude, "that the substance of the corpus luteum is effused between the internal and external membrane of the Graafian vesicle". He apparently was of the opinion that the lutein tissue arose from, or in, the coagulated blood and serum which partially filled the ruptured follicle. His views were not shared by his contemporaries, and in 1842 Bischoff published his studies on material derived from the rabbit, dog, guinea pig and deer. Bischoff proposed the theory that the membrana granulosa persisted after follicular rupture, became hypertrophic, and constituted the mass of cells comprising the yellow body. He found the thecal sheaths playing no significant part in the formation of the corpus luteum.

History then proceeded largely to disregard the conclusions of Paterson and to adopt either the thecal origin of von Baer or the granulosa origin of Bischoff. On the basis of the works of von Baer and Bischoff, there was established in the biological literature a controversy which has persisted to the present day. Many of the more prominent

investigators down through the years have entered the discussion on one side or the other. A completely satisfactory answer to the problem has not, and perhaps never will be written. It is said that a complete list of investigators who have published accounts of the histogenesis of lutein tissue would include some one thousand names. For the present discussion a series of the more important publications will suffice to present the historical background.

With the advantage of several improvements in histological technique, Wilhelm His (1865) first introduced descriptions of specimens which approach those of more recent years. His work included studies of several mammalian forms, Namely: the cow, cat, and man. It is to His that we owe much credit for a complete and concise formulation of the view that the corpus luteum is derived entirely from the inner thecal layer of the ruptured follicle. He ably supported his opinion with a number of acute observations. Among these were the observed facts that the granulosa layer of larger follicles often appears degenerate and may therefore be shed at the time of ovulation. On the other hand, if the follicle should become atretic, the membrana granulosa is seen to disappear by degeneration; while the theca interna was known to undergo hypertrophy and hyperplasia to obliterate more or less the follicular cavity in the formation of an 'interstitial gland', the cells of which then bear a certain morphologic relation to the cells of the mature corpus luteum. Furthermore,His observed with continued ripening of the Graafian follicle

the cells of the theca interna acquire lipoid droplets, increase in size, and in some species accumulate a yellowish pigment, the sum of which processes serves to increase further their similarity to the large lipoid-laden cells of the corpus luteum. This investigation has been criticized on the grounds that His examined only isolated cases and never acquired a complete series of developmental stages in the formation of the corpus luteum.

No serious objections to the investigative work of His appeared in the literature until Sobotta (1895) published the first of an extended series of studies on the histogenesis of the corpus luteum. Sobotta criticized the previous investigators on the ground that they had failed to observe a series of developmental stages in the process of luteinization. Most of them had observed only isolated examples of stages in the formation of corpora lutea, and the majority had drawn their conclusions from material which was poorly timed with reference to the menstrual or estrus cycle, and little was known concerning the age of the corpora lutea. He maintained that it was essential for the investigator to know the habits of his animals well enough to be able to predict ovulation time accurately and to collect a complete series of well timed developmental stages. His material consisted of such a series, and as a further check on the age of the corpora lutea, he relied on the stage of maturation, fertilization, or segmentation, seen in the ova which had been produced by the transforming follicles. In

this manner he was able to arrange his extensive series of stages into a very precisely timed series of developmental stages. Sobotta has been criticized on the basis of his method for the determination of ovulation time in the material he studied. He discovered that the mouse regularly ovulates twenty-one days after parturition. This time interval was so long that the actual hour if not the date of ovulation may be somewhat variable. His series of publications (1895, '96, '97, '99, '01 and '07) nevertheless represents the first of the older group of investigations in which modern histologic methods were employed. As a result of his studies, he was able to establish without question the fact that the membrana granulosa does not degenerate and disappear but persists and is transformed into the typical lutein elements. During this process he observed the cellular theca interna and described the changes occurring there. The lipid-rich thecal cells, lying adjacent to the granulosa layer, were seen to transform themselves slowly into typical spindle-shaped fibroblastic elements, which then proliferated rapidly into the mass of persisting granulosa cells. In this manner the cells of the theca interna were used-up and disappeared without a trace transforming themselves completely into the light stromal network of the fully formed corpus luteum.

Clark (1898) published the results of his studies on a large group of ovaries obtained from swine. He had no information about the material save that many of the animals

being butchered were said to be in heat. He arranged his material as best he could to portray three phases in the life cycle of the corpus luteum, "Ursprung, Wachstung und Ende". He described extensive degeneration in the membrana granulosa of his nearly mature follicles. In these same follicles the theca interna was quite hypertrophic and contained considerable amounts of lipoid material which he called lutein. In other words, the cells of the theca folliculi, which he believed gave rise to the lutein cells of the corpus luteum, actually showed definite evidence of luteinization even before the follicle had ruptured. Clark agreed precisely with von Baer and His in that he concluded that the epithelioid elements of the corpus luteum were derived entirely from the theca interna.

A careful examination of Clark's text and figures is sufficient to lead one to suspect that he described atretic follicles rather than nearly mature normal ones. He did not have serial sections and could not demonstrate the ova in his follicles. It appears that he was actually describing follicular atresia as one stage in the development of the corpus luteum.

In the same year, Rabl (1898), in a paper describing the histology of the ovary of man and several mammalian species, included a discussion of the formation of the corpus luteum. He used human corpora lutea of doubtful age, none of them being really newly-formed, but nevertheless proposed the idea that the cells of the theca interna persist

about the perimeter of the corpus luteum retaining for some time their original characters. They then slowly disappear, some by transforming into typical lutein cells, others by degeneration.

The next investigator to enter the field with a series of papers was Van der Stricht (1901, '08 and '12). His material consisted largely of several species of European bats which he collected in large numbers. He was interested in the development of the embryos as well as the corpora lutea, and he sectioned the ovary, tube and uterus serially. In this way he was able to construct a well timed series of specimens of maturing follicles and corpora lutea. He was able to follow the membrana granulosa through his series without observing any evidence of degeneration in the epithelial cells. On the other hand, the granulosa cells were seen to enlarge significantly, acquire lipoid granules in their cytoplasm, and to transform themselves slowly into the great mass of lutein cells of the yellow body. Van der Stricht also observed the changes occurring in the theca interna during the development of the corpus luteum. He found that shortly after follicular rupture the membrana propria, the basement membrane of the granulosa layer, disappeared, and the granulosa was invaded by spindle cells from the theca interna. These spindle cells, however, were not the distinctive cells of the inner thecal sheath but appeared to be fibroblasts. The distinctive cells of the theca interna he described as remaining in their previous location during the transformation of the granulosa, a few of them

becoming intermingled with the luteinizing granulosa. All of them, he found, eventually became so similar to the granulosa-lutein cells that he could no longer distinguish them. He apparently believed that the lipoid-rich cells of the theca interna became identical to the granulosa-lutein cells and that the corpus luteum is therefore composed of two cell types, which are not distinguishable in the fully-formed structure. Van der Stricht was also able to demonstrate, in his material, definite evidence of mitosis among the granulosa-lutein cells, a fact which had been denied by Sobotta. He believed that the considerable increase in size of the lutein body was largely the result of hypertrophy of the granulosa-lutein cells and partly the result of hyperplasia among the same cells. He considered that the theca interna played a minimal role in the elaboration of the mature corpus luteum.

Jankowski (1904) studied a group of ovaries from swine which he had collected at random. He had no information concerning the ages of his specimens; however, unlike Clark's material, these specimens appear to be normal but represent an incomplete series of developmental stages. He failed to locate the ova in any of his maturing follicles, but the young corpora lutea of his series showed evidence of the presence of the stigma and were probably quite young. On the basis of a few specimens, he agreed with Clark completely since the granulosa in several of his specimens appeared irregular, swollen and contained some vacuoles, he

regarded it as degenerating. On the basis of a few specimens in the same material, he observed large theca interna cells containing a few lipoid granulations and resembling typical lutein cells.

In 1906 L. Loeb found it possible to agree quite closely with the earlier ideas of Van der Stricht. His material consisted of the ovaries from thirty guinea pigs removed at varying intervals following copulation. Loeb thought that the majority of cells of the corpus luteum were derived from the granulosa layer. He felt that a few of the more centrally placed of the granulosa cells degenerated showing vacuolation and karyorrhexis. Others of the granulosa cells appeared to be drawn out toward the stigma; "A number of these may be seen to lose their nuclei and become fibers and stain with eosin". At this time numerous mitotic figures were seen to be present among the interna cells which were becoming hypertrophic (luteinized). He described an intense hyperemia of the theca interna developing shortly after follicular rupture. Along with the hyperemia, there was also an increase in the number of mitotic figures among both the connective tissue cells and the endothelial cells, which were seen to invade the granulosa layer very rapidly during the hyperemic stage. He considered most of the invading spindle-shaped cells to be endothelial and not fibroblastic; however, he admits considerable difficulty in distinguishing between the two during the formative

period. The granulosa and theca layers in some cases remained distinct as long as twenty hours after rupture of the follicle, but shortly thereafter, the two cell types came to resemble each other so closely that it was then impossible to identify them. He observed no evidence of theca cells infiltrating the luteinizing granulosa layer, but stated, his failure to observe any infiltration did not preclude the possibility that the process might occur. He maintained that the mature corpus luteum of the guinea pig was composed of granulosa and theca interna elements which were morphologically and physiologically identical.

Delestre (1910) described the formation of the corpus luteum of the cow based on several specimens of ovarian tissue which he had been able to collect. He had little or no information concerning the relative ages of his corpora lutea and made no attempt to locate or observe the ova. Nevertheless, he believed four of his specimens to be very early corpora lutea, and from his observations of growing and maturing follicles, he concluded that the granulosa layer became degenerate before follicular rupture and that the corpus luteum was formed from the theca interna by hypertrophy and hyperplasia. There seems to be little doubt that Delestre too, confused atresia with follicular maturation and corpus luteum development.

The first controlled study of the histogenesis of the human corpus luteum was published (1911a and b) by R.

Meyer. This study represented observations made upon a series of five human corpora lutea all in the process of development. Careful menstrual histories were available for each specimen, but nothing was known concerning the ova. At this period little was known in reference to ovulation time in the human female, and Meyer could not be certain of their respective ages. One, which he believed to be the earliest human corpus luteum ever to be obtained, was later sharply criticized by several investigators and remains questionable even today. He described four stages in the development and regression of the human corpus luteum. First, a stage of proliferation and hyperemia which was then followed by a stage of glandular metamorphosis and vascularization in which lipid was seen to collect in both the granulosa and theca layers. The stage of maturity or "Bluthezeit", and at length the regression or " Ruckbuildung" appeared.

Meyer described the cells of the theca interna as persisting about the periphery of the ruptured follicle for a variable period of time. He believed that they eventually disappeared by atrophy under pressure of the developing corpus luteum. They appeared to be crowded into the folds of the granulosa layer and sooner or later seemed to be crowded out of existence. It is interesting to note that Meyer was the first to refer to them as theca-lutein cells

O'Donoghue (1912 and 1914) reported his studies on corpus luteum formation in several marsupials. He derived

the corpus luteum from cells of the granulosa layer of ruptured follicles. Like Sobotta, he concluded that the connective tissue stroma of the yellow body was formed by transformation of theca interna cells into the spindle-shaped fibroblastic elements which invaded the mass of luteinizing granulosa cells alongside the capillaries.

In 1915 the first of a long and fruitful series of studies was published by Corner (1915 - 1945) concerned with the development and nature of the corpus luteum of swine and the rhesus monkey. In his first paper, Corner reported observations made on a large number (128 pairs) of ovaries, embryos and fetuses which were collected at the slaughter house. In each case the products of conception were measured, and the relative ages of the corpora were thus recorded. His series contained only a small number of early stages, which number, he considered inadequate to permit him to enter the controversy over the origin of lutein cells. He nevertheless observed and noted the presence of some "additional cells", which appeared to have characteristics similar to those of the theca-lutein cells reported by R. Meyer. In later papers, Corner completed his study of the swine ovary, estrus cycle and, in particular, the corpus luteum of the sow. He became a staunch advocate of the dual origin theory for lutein tissue, describing very carefully the development of lutein cells in the swine both from the theca interna and the granulosa layers. He found no degeneration of the granulosa after rupture of the follicle. The

granulosa cells, on the other hand, were seen to undergo hypertrophy but not hyperplasia; they accumulated considerable amounts of lipoid substances in their cytoplasm and became the larger typical lutein cells of the fully-formed corpus luteum. During this metamorphosis, the granulosa layer was vascularized by capillaries from the theca interna and organized by fibroblasts from the theca externa. While these processes were in progress, the cells of the theca interna increased by mitotic division, lost much of their lipoid inclusions, and migrated into the granulosa or merely remained clustered in the folds of the follicular wall. He could find no evidence that any of the cells of the inner sheath were transformed into spindle cells or fibroblasts. He believed that the theca-lutein cells persisted through pregnancy, some remaining as distinct elements of the lutein body; while others came to resemble quite closely the granulosa derivitives.

More recently, Corner (1923, '40 and '42) and Corner, Bartelmez and Hartman (1936 and '45) have completed a series of studies on the reproductive mechanisms of the rhesus monkey. Included in these are studies on development, organization and breakdown of the corpus luteum and considerable discussion of the corpora aberrantia. Corner et al. have found that the granulosa begins its metamorphosis on the fourth day after ovulation; at about this same time the granulosa layer is invaded by vessels from the theca interna. "Fibroblast-like" cells, believed to be

derived from the capillary endothelium enter the cavity to form the central stromal net. By the seventh to the ninth day, the lutein wall is completely organized and at this time connective tissue begins to appear in the rather dense fibrin network filling the old follicular cavity. During the process of organization the cells of the theca interna are frequently lost to view because of their great similarity to the granulosa lutein cells. In some cases, however, it is possible to follow them through the organization stages; and in all cases, they appear again in the corpus luteum of pregnancy. The theca-lutein or paralutein cells are definitely distinguishable at the bases of the folds and at the periphery of the corpus luteum of pregnancy or in the corpus aberrans. Corner admits the extreme difficulty of following the interna cells through the development phase, but is convinced that they do persist and do become the so called theca-lutein cells of Meyer.

These same investigators have described the existence of two other lutein-like structures in the ovaries of the rhesus monkey. The simplest of these is the accessory corpus luteum which appears to form in unruptured (atretic?) follicles by a process of luteinization of presumably both the granulosa and the theca interna. They were seen to contain in each case evidence of a degenerating ovum. They appeared to represent the same stage of development, bloom or regression as the normal corpus formed at the same time from the follicle which completed its maturation and ruptured. Corner considered that they probably arose at the time

the normal corpus luteum was luteinizing and under the luteinizing influence of the pituitary. The second of these unusual structures was given the name corpus aberrans, and these were finally interpreted as corpora lutea of menstruation, which for some unknown reason perhaps an atypical pituitary influence, continue their existence several months beyond the normal duration. During this time, the granulosa-lutein and theca-lutein cells, of which they were composed, closely resemble those found in the older corpora lutea of preganacy. Corner described three more or less distinct types of corpora aberrantia, and in addition, accessory corpora aberrantia formed from accessory corpora lutea under the same atypical influence. The corpora aberrantia were apparently inactive physiologically; since their existence was not suspected from a careful study of the menstrual histories of the animals prior to sacrifice. The accessory corpora lutea were thought to have been physiologically active since their developemnt ran parallel to that of the normal corpus luteum. This point he considered would be too difficult to determine at the present time.

In 1916 Novak examined a large number of human ovaries (137) in which corpora lutea were found. Menstrual histories were available for all of his cases; but since most of the ovaries were removed for gynecologic reasons, the menstrual histories were not particularly helpful in many cases. He appears to have observed some young specimens and many older ones making a rather complete series.