THE MORPHOLOGY, ANDROGENIC FUNCTION, HYPERPLASIA, AND TUMORS OF THE HUMAN OVARIAN HILUS CELLS *

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The hilus of the human ovary contains nests of cells morphologically identical with testicular Leydig cells, and which, in all probability, produce androgens. Multiple sections through the ovarian hilus and mesovarium will reveal these small nests microscopically in at least 80 per cent of adult ovaries; probably in all adult ovaries if sufficient sections are made. Although they had been noted previously by a number of authors (Aichel,[1] Bucura,[2] and von Winiwarter[3,4]) who failed to recognize their significance, Berger,[5-9] in 1922 and in subsequent years, presented the first sound morphologic studies of the ovarian hilus cells. Nevertheless, there is comparatively little reference to these cells in the American medical literature, and they are not mentioned in standard textbooks of histology, gynecologic pathology, nor in monographs on ovarian tumors (with the exception of Selye's recent "Atlas of Ovarian Tumors"[10]).

The hilus cells are found in clusters along the length of the ovarian hilus and in the adjacent mesovarium. They are, almost without exception, found in contiguity with the nonmyelinated nerves of the hilus, often in intimate relationship to the abundant vascular and lymphatic spaces in this area. Cytologically, a point for point correspondence with the testicular Leydig cells can be established in terms of nuclear and cytoplasmic detail, lipids, lipochrome pigment, and crystalloids of Reinke.

This paper presents a morphologic study of the ovarian hilus cells, a report of 2 cases of masculinizing tumors of these cells, as well as 2 instances of hilus cell hyperplasia associated with masculinization. Additional data concerning the functional significance of the cells and their response to chorionic gonadotropin are recorded.

TERMINOLOGY

A number of names have been applied to the hilus cells. Berger[5-9] referred to them as "sympathicotropic" cells. Kohn[11] used the term "extraglandular interstitial cells" or "extraglandular Leydig cells."

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Neumann,[12] Wieser,[13] and others have referred to them as "hilus cells."

The adjective "sympathicotropic" was applied by Berger because of the almost constant anatomic relationship to the nonmyelinated nerves of the ovarian hilus and mesovarium. However, the term has a physiologic connotation which is perhaps unwarranted in our present state of ignorance. The term "interstitial cell" is likely to be confusing. Interstitial cells of the ovary, apparently derived from theca interna, are prominent in certain animals but there is disagreement as to their existence in humans. In any case they appear unrelated to hilus cells. The term "extraglandular Leydig cell," though a bit cumbersome, has much to commend it. The cells are morphologically identical with Leydig cells and are undoubtedly androgenic. Nevertheless, absolute identity has not been proved; and it may be that the relationship to nerves indicates an additional function, not yet elucidated, which may establish the cells as cousins rather than sisters to the Leydig cells of the testis.

I shall refer to these cells as "ovarian hilus cells." Fortunately, none of the other numerous cell types resident in the ovarian hilus has been so designated. The term suffers, of course, from lack of specificity, but that perhaps may be an advantage at present.

RESUMÉ OF LITERATURE

There are scattered studies of ovarian hilus cells in the earlier literature by authors (Aschoff,[14] Bucura,[2] von Winiwarter,[3] and others) who considered them part of the chromaffin system. Since 1922, Berger,[5-9] in a series of studies, has presented convincing morphologic evidence of the similarity of these cells to testicular Leydig cells, referring to them as sympathicotropic cells. A lively controversy between the proponents of these two theories existed for many years. Reference may be made to the papers of de Winiwarter,[15-17] the stoutest proponent of the "paraganglion cell" theory, as well as to those of Wallart[18] and Joachimovits.[19] Since Berger's initial studies, most investigators have agreed with his conclusions (Kohn,[11] Pawlowski,[20] Brannon,[21] Wieser,[13] Barrozo do Amaral[22]).

Particular reference is made to the study by Kohn[11] (who, incidentally, discovered the pheochrome system). After a morphologic analysis he rejected the possibility that the ovarian hilus cell belongs to the chromaffin system. Most investigators, including myself, have been unable to demonstrate a chromaffin reaction in these cells. It is altogether likely that some who have reported chromaffin staining were misled by the brown lipochrome pigment normally present in many of the cells.

In the human newborn the identification of cells adjacent to the ovary is complicated, since true pheochrome cells also may be present, particularly in the broad ligament. Chromaffin tissue in the embryo, after separating from the sympathico-chromaffin anlagen, may be closely associated with the wolffian body. Zuckerkandl[23] noted that chromaffin tissue may be found in the fetus and newborn along the ureters, in the broad ligaments, retroperitoneal tissues, and elsewhere. However, these nests are morphologically distinct from the hilus cells and do show a chromaffin reaction. Berger[7] has reported the simultaneous presence of cells of both types in the newborn. Pheochrome nests about the ovary, however, are quite rare in the adult since in the human there is a general involution of chromaffin tissues shortly after birth.

Cell nests identical with the ovarian hilus cells have long been known to exist in the male gonad outside of the testis proper. Verocay,[24] in 1915, first studied these, and they have since been noted by numerous authors.[25,26] They may be found in the tunica albuginea, the hilus of the testis, the region of the rete testis, along the spermatic cord, and elsewhere. They have, furthermore, precisely the same relationship to nonmyelinated nerves as do ovarian hilus cells, and in fact are histologically indistinguishable from them. Berger[5,8] has studied these extratesticular Leydig cells extensively, as have Wieser,[13] Kohn,[11] Nelson,[27] and others.

Kohn[11] studied ovarian hilus cells in the newborn, and noted their prominence in an anencephalic infant. He found them as late as the 77th year of life. Brannon,[21] who studied a large series of ovaries, found abundant hilus cells in ovaries of 4 pregnant women, all cases of toxemia.

Morphology of Ovarian Hilus Cells

Over 100 ovaries (singly or in pairs) taken from routine surgical and autopsy material were studied. Since the hilus cells cannot be distinguished grossly and since they are distributed in irregularly scattered clumps, they may be absent from some sections cut through the hilus of an ovary but present in others. However, using a limited number of sections (4 to 6, on an average) hilus cells were identified in approximately 80 per cent of the adult ovaries studied. In the ovaries of 12 adult women a more thorough search was made and hilus cells were found in all cases. It is altogether probable that some of these cells are present in all adult ovaries. In general more hilus cells were found per section when the ovarian hilus and mesovarium were sectioned longitudinally after fixation.

DISTRIBUTION

There was considerable variation both in the number and distribution of the ovarian hilus cells. They were present in the hilus and mesovarium, being somewhat more numerous at the lateral and medial poles of the hilus, and particularly near the junction of the ovarian ligament and ovary. Cell nests were seen frequently in the areolar tissue of the hilus close to its well defined junction with the ovarian stroma. However, nests were seen often much deeper in the mesovarium. Cell nests might be found close to the rete ovarii although no constant anatomic relationship was apparent. Occasional nests were incorporated in the ovarian stroma, but these were almost always close to the hilus and appeared to represent extensions of the hilus into the ovary proper.

The cells occurred in aggregates that varied considerably in size, shape, and number of cells. Irregularly shaped cell clusters often partially surrounded vessels and nerves in the vicinity. Unlike the occasional adrenal rests of the region, they never were encapsulated or delimited sharply from the surrounding tissues, but rather seemed indigenous to the hilar stroma.

CYTOLOGY

The remarkable range of morphologic variations in the testicular Leydig cells is well known to all who have studied them. They vary from cells indistinguishable from the fibroblast, through elongate, polygonal, and oval forms containing an inconstant variety of intracytoplasmic inclusions, to rare giant multinucleated types. This protean variation in form might appear to offer difficulties in establishing the morphologic identity of hilus and Leydig cells. Actually, the reverse is the case, since the ovarian hilus cells reveal precisely the same types of morphologic variation.

The majority of ovarian hilus cells measured from 14 to 25 μ in diameter, although occasional larger forms were seen. Frequently each cell in a group was separated from its neighbor by a delicate collagenous fibril, as seen often with testicular Leydig cells. The cells for the most part were polygonal or oval although occasional elongate forms were seen.

The nucleus was vesicular and more constant morphologically than the cytoplasm. It was spherical, averaging 7 to 10 μ in diameter; occasionally it was ovoid or reniform. The chromatin clumps were coarse and rather sparse. Most cells contained one or two basophilic oval nucleoli. Occasionally three nucleoli were seen.

The cytoplasm was acidophilic, with a well marked granularity. A

narrow clear zone often was seen about the nucleus. Many cells were vacuolated but the degree of vacuolization was highly variable. Some cells had a zone of acidophilic granulation about the nucleus and vacuoles in the peripheral cytoplasm, an arrangement common in testicular Leydig cells. In one area the cells might be of smaller average size with a deep acidophilic and nonvacuolated cytoplasm and a darker nucleus, while adjacent areas might show larger cells with paler, more abundant cytoplasm and a clearer nucleus. The latter cells were more likely to show lipid vacuolization. These cell types may represent variations in the state of secretion of the cells.

Nelson[27] found giant multinucleated interstitial cells in 85 of 721 testes studied. Identical forms were found occasionally among the hilus cells. A group of these giant multinucleated ovarian hilus cells is illustrated in Figure 20.

Cytoplasmic Inclusions

Lipids. The cytoplasmic vacuoles contained lipids which stained brilliantly with the usual fat stains such as sudan III. As with testicular Leydig cells, the lipid content varied considerably from one cell to another. No stainable lipid was present in some cells; others might show only fine dust-like, sudanophilic droplets; and in some the cytoplasm was filled with large, irregular masses of sudanophilic material. Some, but not all, of the lipid droplets were anisotropic.

Pigment. Small round granules of golden-brown pigment were present in the cytoplasm of some cells, in both stained and unstained sections. The pigment granules took up sudan III to some extent. This pigment probably belongs to the group of lipochromes, and is identical in appearance with the lipochrome of Leydig cells. As in the testis, it is more prominent in older patients.

Crystalloids of Reinke. Testicular Leydig cells in human beings may contain characteristic "crystalloidal" cytoplasmic inclusions. These curious structures were first described by Reinke[28] in 1896 and, although nothing has been learned of their function, they are generally accepted as structures peculiar to Leydig cells. However, they are found also in the ovarian hilus cells, a fact which greatly strengthens the morphologic evidence of identity.

The crystalloids of Reinke are acidophilic rod-like structures within the cytoplasm. They are often as long as the greatest diameter of the cell, at times measuring 30 μ, more commonly averaging 10 to 20 μ. The ends are rounded or rectangular with blunted corners. Often a straight, clear line bisects the structure longitudinally. Commonly a clear zone or halo surrounds the structure. They are frequently multiple in

a given cell, tending to a parallel arrangement. Smaller, narrower types are found which have tapering or pointed rather than blunt ends. At times the crystalloids appear to be extruded partially from the cytoplasm of the cell.

The crystalloids were found in only a small percentage of hilus cells (as is the case with testicular Leydig cells) and patient search was necessary. In many ovaries, even with the study of a great many sections, they were not found. When present, they usually had a patchy distribution, abundant in localized areas and absent in adjacent clusters of hilus cells. When a single crystalloid was seen, others generally were found in neighboring cells. The significance of this distribution is not understood. Crystalloids in abundance and with the same patchy distribution were found in both of the cases of masculinizing tumors to be described.

What little histochemical data are available suggest that the crystalloids are protein bodies. They take the acid dyes strongly, are not sudanophilic, not doubly refractile, nor do they dissolve in the fat solvents. Eberth[29] stated that they swell in 10 per cent potassium hydroxide and dissolve in hydrochloric acid with pepsin, although they are insoluble in 10 per cent hydrochloric acid, nitric acid, and acetic acid.

I have observed spherical acidophilic bodies of varying size in the cytoplasm of cells in close proximity with crystalloid-containing cells. These "hyaline" spheres had the same staining reactions as the crystalloids, and may represent precursors of them. They were particularly prominent in the tumor cells in case 2 where crystalloids also were very abundant. In some fields the majority of cells which did not contain crystalloids contained these bodies. Often they occupied most of the cytoplasm of the cell, at times compressing the nucleus to one pole. Structures that appeared to be transitional between the spherical bodies and the crystalloids were present. These intermediate forms were ellipsoids, or broad rectangles with rounded corners. Some are illustrated in the camera lucida drawing (Fig. 23). These observations suggest that the typical crystalloids may develop by gradual transformation from pre-existing spherical cytoplasmic inclusions.

RELATIONSHIP TO NERVES

There was a constant relationship of ovarian hilus cells to the nonmyelinated nerves of the hilus. The cells were commonly found in masses irregularly ensheathing nerves. Even more striking was the presence of hilus cells, scattered singly or in groups, within a nerve trunk, lying between individual nonmyelinated nerve fibers and often producing localized bulging of the contour of the nerve. When nests were found

apparently isolated from nerves, serial sections almost always established contiguity with a nerve. I have confirmed the observations of Berger[5,8] and others that Leydig cells, located outside of the testis proper, have precisely the same relationship to nerve. In one instance, I also have observed a nonmyelinated nerve bundle within the testis proper, in which Leydig cells were dispersed in similar fashion within the nerve, separating adjacent nerve fibers.

RELATIONSHIP TO VESSELS

The ovarian hilus cells and their associated nerves often bear an intimate relationship to the vascular spaces of the ovarian hilus and mesovarium. The normal ovarian hilus has a remarkably complex vascular structure which has never been adequately explained. The bulk of the hilar tissue is made up of a tangled meshwork of small, tortuous, muscular arteries, large venous sinuses, and lymphatic spaces. Its vascular structure has recently been emphasized by the excellent injection preparations of Reynolds.[30] The number, size, and concentration of vessels seem far in excess of any reasonable vascular needs of so small an organ as the ovary.

Masses of hilus cells and nerve containing them were found in close association with large venous and lymphatic sinusoids. In fact, nerves containing or surrounded by hilus cells often formed distinct nodular protrusions into the lumina of vessels. In such areas the lumen appeared to be separated from the perineurium only by a layer of endothelial cells. Such structures are illustrated in Figures 7 and 8.

HYPERPLASIA OF HILUS CELLS ASSOCIATED WITH MASCULINIZATION

Two instances of hyperplasia of ovarian hilus cells associated with clinical masculinization may be mentioned briefly. Both cases will be reported in greater detail in a subsequent study.

The first of these, Mrs. R. M., a 41-year-old para II, gravida II, had shown masculinization for 8 years. Among the pertinent findings were a heavy beard requiring daily shaving, a deep voice, large clitoris, and a masculine, although somewhat obese, habitus. Menses occurred about every 2 months.

The second case, Mrs. V. P., a 50-year-old para I, gravida II, gave a history of masculinization of 3 years' duration. Previously a well developed female, she grew a heavy beard, and her voice deepened. Menstrual periods, which had been regular, ceased 5 months before admission. Pertinent findings included obesity of abdomen, thighs, and hips, hirsutism of face and body, an enlarged clitoris, and atrophic breasts.

Both patients were subjected to abdominal exploration and panhysterectomy. The adrenals in both patients were normal grossly at operation.

Tissue taken for biopsy of an adrenal in the first case was not remarkable. In both patients the ovaries were eight to ten times the normal

size for the age, and were fairly firm. Except for a single small follicular cyst in the first case, no cysts or follicles were present. Microscopic examination revealed field after field of ovarian stroma with only several corpora albicantia interspersed. Of greatest interest, however, was the presence in both cases of a significant increase in the number of ovarian hilus cells.

Rare instances of greatly enlarged ovaries associated with masculinization are described in the literature.[31-34] No very satisfactory explanation of the masculinization in such cases has been proposed, although "hyperthecosis"[33,34] of the ovaries, and pituitary stimulation[32] have been advanced. It seems likely that the hyperplasia of ovarian hilus cells, in the 2 cases cited, was largely responsible for the development of masculinization. Whether this increase of hilus cells was secondary to other hormonal stimulation cannot very well be discussed within the scope of this paper. The significance of the great increase in bulk of the ovarian stroma is obscure. In this regard, however, the relatively large size of the ovaries in the case with bilateral hilus cell tumors, presented below, is worth emphasizing.

Hilus Cell Tumors with Masculinization

Berger,[8] in 1942, reported a case of masculinization associated with a small tumor of ovarian hilus cells. The patient, 50 years of age, had been masculinized for 18 years. There was facial hypertrichosis requiring shaving two or three times weekly, a masculine body build, deep voice with a prominent larynx, atrophy of the breasts, and an enlarged clitoris. In spite of these secondary sexual changes, she menstruated regularly. Following removal of ovaries, uterus, and tubes there was a significant regression of her masculinization. In the medial portion of one mesovarium there was a small tumor measuring 4.5 by 3.5 mm. composed of ovarian hilus cells. This is the only definite account of such a tumor which was found in the literature.

Another possible example of a similar neoplasm reported by Dreyfus and Barrozo do Amaral[34a] occurred in a female pseudo-hermaphrodite, but the picture was complicated by the simultaneous presence of aberrant corticoadrenal tumors in the region of the adrenal gland.

The following 2 examples of ovarian hilus cell tumors associated with clinical masculinization bring the total number of established cases to 3.* It is likely that others exist but have not been accurately diagnosed.

* Since this paper was submitted for publication, I have observed one additional case of hilus cell tumor, as well as 2 instances of hilus cell hyperplasia, all 3 associated with masculinizing syndromes.

Case 1

Clinical History. Mrs. C. G., a masculinized colored female, 86 years of age, was admitted to the Tulane Service of Charity Hospital on April 21, 1947, with a complaint of vaginal discharge and vague abdominal pain present for several months. Her childhood had not been remarkable and female characteristics had developed normally. She had borne three full-term children, all of whom had died in early infancy. Evidence of masculinization, as well as could be determined, dated from her 54th year, when she first began to grow a beard. Some time later her voice deepened. Because of her difficulty in recalling past events, little is known of her menstrual history, save that she had not menstruated nor had any vaginal bleeding for many years. No members of her family showed evidence of masculinization.

Physical Examination. Her features and habitus simulated those of an elderly male to a remarkable degree. She looked distinctly younger than her stated age and her skeletal musculature was remarkably well developed. She weighed 110 lbs.; her height was 136 cm. Her blood pressure varied between 140/88 and 164/100 mm. of Hg; pulse, between 58 and 70. She had a heavy mustache and a coarse, straggly beard. Her features were coarse and there was recession of the hairline at the forehead. The thyroid was not palpably enlarged. The thyroid cartilage was prominent and her voice deep. The breasts were small and atrophic and the nipples flat. A hard, nodular, nontender, movable mass was palpable in the lower abdomen, apparently arising within the pelvis and extending several centimeters above the symphysis. A male distribution of pubic hair was present; the labia majora and minora were atrophic. The clitoris was greatly enlarged, measuring 3.5 cm. in length by 1.5 cm. in diameter. The vaginal mucosa was not as atrophic as one might have predicted from the vulvar atrophy. The cervix was small and clean. Three or four stony masses projected from the sides of the uterus. The adnexae were not clearly outlined but it was felt that both ovaries were slightly enlarged and firm.

Laboratory Studies. Routine examinations of blood and urine were normal. Urinary concentration was good. The glucose tolerance test revealed a fasting level of 100 mg.; ½ hour, 135 mg.; 1 hour, 143 mg.; 2 hours, 103 mg.; 3 hours, 100 mg., per 100 cc. of blood. Twenty-four hour urinary 17-ketosteroids, as determined on three occasions by Dr. Albert Segaloff, were 6 mg., 9 mg., and 6 mg. The basal metabolic rate was —16.

Roentgenograms of the skull were normal, but those of the abdomen revealed multiple large areas of calcification in the lower abdomen and pelvis, compatible with calcified leiomyomas. Intravenous pyelograms were not remarkable.

The patient was considered a good surgical risk despite her age, and on May 1, 1947, was operated upon on the Gynecology Service. The adrenals were palpated at operation and were not enlarged. The ovaries, although of normal contour, were moderately enlarged for a woman of 86 years and the uterus was distorted by several partially calcified leiomyomas. A total hysterectomy and bilateral salpingo-oophorectomy were done. The postoperative course was uneventful save for a wound infection and she was discharged on June 20, 1947, in excellent health.

Pathologic Examination

The specimen consisted of an enlarged uterus with both tubes and ovaries attached. The cervical canal was stenotic for 2 mm. in the region of the external os. The uterus was grossly distorted by numerous leiomyomas, many of which were calcified. The endometrial cavity measured 6.5 cm. in length and 4 cm. in its greatest width. The endometrium was

pale pink, measuring less than 1 mm. in thickness. The tubes were not remarkable.

The ovaries were distinctly larger than would be expected from the advanced age of the patient. They corresponded roughly to ovaries of the 45 to 50 year age group, each measuring 3.5 by 2.5 by 2 cm. The serosal surfaces of both ovaries showed the usual pitting and corrugations. The ovarian tissue was firm but retained a slightly fleshy consistency. It was pinkish gray on section, with an indistinct tannish mottling. There were several corpora albicantia but no cystic structures present.

At the uterine pole of each ovarian hilus, lying within the mesovarium but contiguous with the ovarian stroma adjacent to the ovarian ligament, was a small, clearly demarcated tumor mass. The two tumors were remarkably symmetric in both position and size. They varied from a dark orange-brown to a dark olive-brown, and were of homogeneous fleshy consistency. The tumor at the left hilus measured 1 by 0.7 by 0.5 cm.; the tumor at the right hilus measured 1.2 by 0.8 by 0.5 cm.

Histologic Examination. The endometrium showed senile atrophy with cystic dilatation of the endometrial glands. The myometrium was atrophic. The leiomyomata showed considerable hyaline degeneration with extensive zones of calcification in some. There was chronic endocervicitis and dilatation of the endocervical glands. The tubes were not remarkable save for senile atrophic changes.

The ovaries did not show the advanced senile atrophy of the stroma expected in a woman of 86 years. The ovarian stroma was moderately cellular, comparable to that in the age group of 45 to 50 years. The tunica albuginea was of average thickness. A number of corpora albicantia were present. There were no follicular, thecal, or lutein cellular elements. Along the entire length of the ovarian hilus and adjacent mesovarium in both ovaries there was a striking abundance of hilus cell nests. The hilus cells showed the usual morphologic variations but were greatly increased in number. The usual relationship of hilus cell nests to nerves and vascular spaces was observed. Occasional crystalloids of Reinke were present within the cytoplasm of hilus cells. Figure 14 is a diagrammatic representation of the left ovary and adnexa as seen from behind.

Histology of Tumors. The bilateral tumors were composed exclusively of ovarian hilus cells, with a sparse accompanying stroma. They were not encapsulated. Occasional nonmyelinated nerve bundles lay adjacent to the periphery of the tumors and in some instances partially penetrated them. Neighboring hilus cells extended along the nerves so

that it was difficult in some regions to differentiate tumor cells from the hilus cells of the vicinity.

The two tumors were composed of well differentiated cells, many of which were indistinguishable from normal hilus cells. A considerable number, however, were larger, many measuring two and three times the diameter of average hilus cells. There was a greater variation in size and form in the tumor cells than in normal hilus cells. Many of the nuclei were irregularly shaped, but were otherwise comparable to the nuclei of hilus cells. Particularly toward the periphery of the tumors, the cells assumed elongate forms. A variable quantity of collagenous stroma was present in different parts of the tumor. Small groups of cells and often individual cells were surrounded by delicate collagenous fibrils. The cytoplasmic structures and staining reactions were identical with those of normal hilus cells.

Crystalloids of Reinke were abundant in the tumor cells, but the distribution was patchy, some areas containing numerous crystalloids, others being free of them. The crystalloids were mostly of the large variety, although occasional cells contained aggregates of small crystalloids. They were particularly well stained with the Masson trichrome stain and with phosphotungstic acid hematoxylin.

The cytoplasm was distinctly granular and acidophilic. Many cells showed marked granularity about the nucleus with vacuolization of the peripheral cytoplasm. With acid fuchsin, somewhat larger granules, 1 to 2 μ in diameter and lying within vacuoles, stained brightly. These were more prominent in the periphery of the cytoplasm. They correspond to the granules described by Whitehead[35] in his study of Leydig cells.

Mitochondria in the tumor cells were stained with phosphotungstic acid hematoxylin after preliminary mordanting of formalin-fixed material in 5 per cent aqueous ferric chloride after the method of Mallory. The mitochondria stained a deep blue. They were abundant in the form of round granules and short rods, duplicating the mitochondrial stains illustrated by von Winiwarter[36] in testicular Leydig cells. Some cells showed larger irregular clumps, small ring forms, and irregular networks in the peripheral cytoplasm. These probably represented mitochondria distorted by fixation as suggested by Duesberg[37] for the Leydig cells. They corresponded very closely to Duesberg's illustrations.

Many of the cells contained yellow-brown lipochrome pigment in small round granules in the cytoplasm, which probably accounted for the brownish color of the tumors. Sudan III stains of frozen sections revealed abundant but irregularly distributed lipid. Some cells were

free of sudanophilic material; the more vacuolated cells contained large amounts in the cytoplasm. Other cells contained only sparse small droplets of sudanophilic material. The lipochrome pigment took the stain to some extent. The variation in lipid content was comparable to that in Leydig cells. Some of the stainable lipid was doubly refractile when examined with the polarizing microscope.

Case 2

Clinical History. Mrs. E. W., a white woman, 64 years old, was admitted to the Tulane Medical Service of Charity Hospital on April 18, 1947. During the previous 2 years she had developed a beard and mustache, as well as increased hair on the arms and legs. Beard growth averaged ¼ inch weekly. During this period her voice had deepened. Two years previously she had been a normal female. She had borne two normal full-term children, now 35 and 33 years old. Her family history was not contributory. Her menstrual periods, which had been regular, ceased at the age of 40. There had been no subsequent vaginal bleeding except for an episode of spotting 8 months before admission.

Physical Examination. The patient was short, moderately obese, and weighed 146 lbs. There was congenital absence of the left arm below the elbow. The important findings included a marked facial hirsutism, and less marked hirsutism of the arms and legs. There was some recession of the hairline at the forehead. The pubic hair showed a tendency toward masculine distribution. The features were heavy and appeared masculine. The thyroid cartilage was slightly enlarged. Breasts and abdomen were obese. The blood pressure on different occasions varied between 110/60 and 146/96 mm. of Hg. On pelvic examination the clitoris was not grossly enlarged. A senile vaginitis was present, and there was a second degree cystocele. The uterus and adnexae were not definitely outlined.

Laboratory Findings. Results of routine examinations of blood and urine were within normal limits. A glucose tolerance test showed a fasting blood sugar of 103 mg.; ½ hour, 167 mg.; 1 hour, 121 mg.; 2 hours, 103 mg.; 3 hours, 105 mg., per 100 cc. of blood. Basal metabolic rates on three occasions were +16, +16, and +25. The Kline and Kolmer tests were negative. Roentgenograms of the skull and chest and retrograde pyelograms were not contributory. Determination of the 24-hour urinary 17-ketosteroids by Dr. Albert Segaloff showed 7.5 mg.

The patient was transferred to the Tulane Gynecology Service and a bilateral salpingo-oophorectomy and supracervical hysterectomy were performed on May 22, 1947. At operation the adrenals were not palpably enlarged. Her postoperative course was uneventful.

When seen 2 months after operation she complained of occasional hot flashes. Eight months after operation her voice had resumed a normal female pitch and there was a definite reduction in facial hirsutism.

Pathologic Examination

The uterus, removed supracervically, was of average size and contained no leiomyomas. The uterine cavity was small, with a thin endometrium. The tubes, except for slight thickening of the walls, were not remarkable.

The left ovary measured 2.8 by 1.3 by 1 cm.; the right ovary, 2.5 by 1.5 by 1.5 cm. Both were firm and grayish with the usual surface cor-

rugations. On section the ovarian stroma was grayish tan and free of cysts. In the inner medullary portion of the right ovary, midway between the poles, there was a spherical, yellow tumor nodule measuring 1 cm. in diameter. It was unencapsulated and fleshy. The tumor was surrounded by ovarian stroma for the most part, except for a portion which extended into the hilus region and adjacent mesovarium.

Histologic Examination. The endometrium was atrophic, with cystic dilatation of the endometrial glands. Moderate adenomyosis was present. The tubes showed healed chronic salpingitis.

The ovaries contained multiple corpora albicantia. The ovarian stroma showed moderate fibrosis and atrophy compatible with the age of the patient. The hilus cells in both right and left mesovaria were moderately increased in number although there was less hyperplasia than in case 1. Crystalloids of Reinke were fairly numerous. Nests of hilus cells lay in close proximity to the portion of tumor within the mesovarium. However, these were separated from the tumor, which was sharply circumscribed although not encapsulated.

Histology of Tumor. Sections of the tumor were prepared with hematoxylin and eosin, Masson trichrome, and sudan III stains. The histologic findings were very similar to those of case 1. The tumor was composed of well differentiated hilus cells, polyhedral and somewhat larger than average hilus cells. Mitotic figures were seen rarely. Few cells of the elongated form noted in case 1 were present, and the stroma was sparse and delicate. Nuclear and cytoplasmic details showed the same variations. Lipochrome pigment was present but less abundant. Stainable lipid was similar in amount and distribution to that in the tumors of case 1. Some of the lipid was doubly refractile.

Crystalloids of Reinke were present in even greater abundance than in case 1. Most of these were of the large type, some measuring as much as 35 μ in length. Occasional aggregates of smaller crystalloids were present also. As previously noted, numerous cells containing round acidophilic bodies with the same staining reactions as crystalloids were present. These may represent precursors of crystalloids. They were noted also in the hilus cells outside of the tumor and were more abundant than in case 1.

EVIDENCE OF FUNCTIONAL ACTIVITY OF OVARIAN HILUS CELLS

In these 4 patients with masculinization and with tumors or hyperplasia of hilus cells, the evidence is reasonably good that the hilus cells were functionally important. Do the ovarian hilus cells in the normal adult female produce androgens or do they merely represent vestigial structures? A precise answer is not possible in the present

state of our knowledge. The following facts, however, suggest that the cells normally have a secretory function.

The ovarian hilus cells are present at birth and can be identified during the first year or so of life. Following this and until the age of puberty they are absent or at least difficult to find. They reappear at puberty and persist through adult life, tending to decline in old age.[13] They are particularly prominent during pregnancy and at the menopause. These chronologic relationships suggest functional activity.

Furthermore, the cells have the histologic appearance of actively secreting cells. Cytoplasmic granules and lipid-containing vacuoles are prominent as they are in testicular Leydig cells. The histologic variations in the cells, as in testicular Leydig cells, suggest a life cycle of secretory activity. Crystalloids of Reinke in testicular Leydig cells do not appear until the age of puberty, when the cells become active. The only instance that I have seen of crystalloids of Reinke in the testes of a child occurred in a 4-year-old boy with precocious puberty of so-called constitutional or idiopathic type. Spermatogenesis was active and the Leydig cells were well developed, containing abundant crystalloids. Although the precise importance of the crystalloids is unknown, they appear to be associated (although inconstantly) with functional activity of the cells. The presence of crystalloids, then, in the hilus cells of normal adult women may reflect their secretory activity.

Finally, I should like to present evidence that the ovarian hilus cells respond to stimulation by chorionic gonadotropin. Ovaries from patients who had received 10,000 units of purified chorionic gonadotropin daily for at least 2 weeks were studied histologically.* Injections were begun shortly after the estimated time of ovulation. At this dosage, as reported by Brown and Bradbury,[38] menstruation may be delayed for 7 or more days. Significant changes were noted in the ovarian hilus cells in the 3 cases studied thus far. In all, the hilus cells were abundant and showed a severe though patchy degeneration (Fig. 10). In such areas the cytoplasm was dense and deeply acidophilic, cell boundaries were lost so that adjacent cells appeared to fuse together, and the nuclei were distorted and pyknotic, with a tendency for adjacent nuclei to clump together. Hilus cells in other areas were normal in appearance. A similar degenerative change in hilus cells was noted in the opposite ovary in a case of teratoma in which urinary gonadotropin was elevated. Brannon[21] reported similar degenerative changes in the hilus cells of a woman dying of pernicious vomiting of pregnancy. The possi-

* I am indebted to Drs. Brown and Bradbury for supplying me with ovarian tissue and adjacent mesovarium from one of their cases.

bility that the changes which he noted were due to chorionic gonadotropin stimulation must be considered.

Of particular interest was the presence of mitotic figures in the hilus cells of one case receiving large doses of chorionic gonadotropin. This case also showed degenerative changes in other groups of hilus cells. Mitotic figures are so rare in normal cells that none was found in over 100 routine specimens examined, although rare mitotic figures were present in the hilus cell tumors. Mitotic figures are equally rare in testicular Leydig cells.[39] The presence, then, of significant numbers of mitotic figures in the hilus cells of a patient who received chorionic gonadotropin is probably not fortuitous.

The evaluation of these morphologic changes in hilus cells with chorionic gonadotropin is not simple. However, as is well known, chorionic gonadotropin stimulates the growth and secretion of Leydig cells in the male. It is possible that the degenerative changes in the hilus cells are an exhaustion effect, particularly since the dosage of gonadotropin used was considerable. Nevertheless, the fact that hilus cells show responses to hormonal stimulation is additional evidence suggesting functional activity.

Discussion

Specific tumors of ovarian hilus cells are rare, as are ovarian masculinizing tumors in general. The case reported by Berger[8] and the two here presented are in fact the only established cases in the literature. It seems altogether likely, however, that some hilus cell tumors have been misdiagnosed as arrhenoblastomas, adrenal rest tumors, or luteomas, especially since the specific diagnosis of the masculinizing ovarian tumors is frequently a source of controversy among pathologists. Critical study of several reports of atypical masculinizing ovarian tumors strongly suggests that some of them may represent hilus cell tumors.

Clearly the hilus cell tumors should be separated from the broad and rather loose category of arrhenoblastomas. In the hilus cell tumors one finds neither the differentiated tubular structures nor the undifferentiated sarcoma-like pattern of the arrhenoblastoma. Unlike the more complex arrhenoblastoma, the hilus cell tumor represents a benign, well differentiated neoplasm composed of cells of a single type, which is similar to that of cells present in the normal ovarian hilus.

Although it is possible that tumors of hilus cells could be confused with adrenal rest tumors, there should be little difficulty in distinguishing adrenal rests of the ovarian hilus from hilus cell nests. The adrenal rests are usually yellow, spherical nodules, 1 to 3 mm. in diameter, sharply circumscribed and encapsulated. The hilus cells, in contrast, are

never encapsulated and tend to be dispersed in many small, irregular nests within the connective tissue of the hilus. Furthermore, the adrenal rests have an organoid structure, with cell cords centripetally arranged and duplicating normal adrenal cortex. Usually an indication of adrenal cortical zones is recognizable. The cells of the zona fasciculata are crowded with uniformly dispersed lipid-containing vacuoles, unlike the irregular and sparser lipid vacuoles of hilus cells. During the past year, we have seen in our laboratory at least six adrenal rests located in tissues adjacent to the adult ovary, as well as comparable structures adjacent to the testes in newborns. They are not likely to be confused with hilus cell nests. A typical example of an adrenal rest in an ovarian hilus is illustrated in Figure 25.

Certain aspects of the three known instances of masculinizing hilus cell tumors are worthy of comment, although generalizations are impossible on so small a group. In all, the tumors were of small size, 1 cm. or less in diameter. They were composed exclusively of ovarian hilus cells, well differentiated and apparently entirely benign. Crystalloids of Reinke, which, when present, are the most convincing identifying feature of these cells, were abundant in the tumors reported here. They were not present in Berger's case, nor need they be considered as a constant finding since they are present in only a fraction of normal hilus cells.

Excretion of urinary 17-ketosteroids in the two cases reported here were within normal limits, as may also be the case in women with masculinization due to arrhenoblastoma.[40,41] In contrast, 17-ketosteroids are more commonly elevated in masculinization due to adrenal lesions.

The persistence of menses in Berger's case and the lack of enlargement of the clitoris in case 2 indicate that the masculinizing syndrome need not be complete. The absence of hypertrophy of the clitoris in the latter case may reflect a difference in end-organ response to hormonal stimulation. Women treated with fairly large doses of testosterone for metastatic breast carcinoma show a striking difference in clitoris response, ranging from lack of detectable growth to marked hypertrophy.[42]

It is now well established that the normal adult female produces androgens. There is, in fact, but little difference either qualitatively or quantitatively in the urinary excretion of 17-ketosteroids in males and females.[43,44] The source of androgens in the female is generally considered to be the adrenal cortex. There is, however, considerable experimental evidence that the ovary, too, is capable of elaborating male sex hormones.[45-49] The specific cell producing these ovarian androgens is not clearly established although some investigators implicate lutein cells or lutein-like cells. It seems likely that in the human, one source of

androgen is the ovarian hilus cell, although its quantitative importance (compared, let us say, to the adrenal) is difficult to evaluate. The morphologic identity of the hilus cells with Leydig cells, their cytologic characteristics as secreting cells, their response to chorionic gonadotropin, and the evidence of masculinization with either tumors or hyperplasia of these cells all point to androgen production.

Further histochemical and physiologic investigations are needed to clarify the rôle of these cells in the normal female. The relationship of the hilus cells to sympathetic nerves and vascular spaces seems too striking to be fortuitous. That these anatomic relations may reflect some special physiologic mechanisms is an intriguing possibility worthy of further investigation.

SUMMARY

Cells morphologically identical with testicular Leydig cells are regularly found in the human ovarian hilus and mesovarium. These ovarian hilus cells have a constant and intimate relationship to nonmyelinated nerves and vascular spaces. A similar relationship of Leydig cells to nerves and vessels is seen in the testicular hilus and adjacent structures.

Morphologic evidence, including the prominence of these cells at puberty, during pregnancy, and at the menopause, suggests functional activity. There is also evidence that they are responsive to stimulation by chorionic gonadotropin.

In the 2 cases of specific tumors of these cells and 2 cases of hyperplasia which are reported, there was masculinization.

The ovarian hilus cells may represent a further source of androgen in the normal female in addition to that derived from the adrenal cortex.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Two typical crystalloids of Reinke in the Leydig cells of the testis of a boy, 4 years old, with precocious puberty of idiopathic or constitutional type. Such crystalloids are a normal finding in mature testes. Crystalloids are seen in the lower left field; seminiferous tubules, above and lower right. (Slide was provided through the courtesy of Dr. Albert Segaloff.) Masson's trichrome stain. × 500.

FIG. 2. Ovarian hilus cells with abundant crystalloids of Reinke from a normal female, 39 years old, operated upon for uterine leiomyomas. Of note are the granular and vacuolated cytoplasm and acidophilic crystalloids. Hematoxylin and eosin stain. × 580.

FIG. 3. Ovarian hilus cells within and adjacent to a nonmyelinated nerve trunk of the mesovarium, a typical picture that may be seen in the hilus of normal ovaries. This is from the ovarian hilus at some distance from the hilus cell tumor in case 1. Hematoxylin and eosin stain. × 170.

FIG. 4. Hilus cell tumor in case 2, a 64-year-old masculinized female, showing large crystalloids of Reinke in tumor cells. Hematoxylin and eosin stain. × 670.

FIG. 5. Hilus cell tumor in case 1, an 86-year-old masculinized female, showing a variable distribution of sudanophilic substance in the cytoplasm of the cells. Frozen section: sudan III and hematoxylin stains. × 500.

FIG. 6. Hilus cell tumor in case 1, an 86-year-old masculinized female. Several crystalloids of Reinke are seen in one cell. The cells are larger than normal hilus cells, and the cytoplasm is abundant. A delicate collagenous fibril surrounds each cell. The brown pigment is lipochrome pigment. Masson's trichrome stain. × 450.

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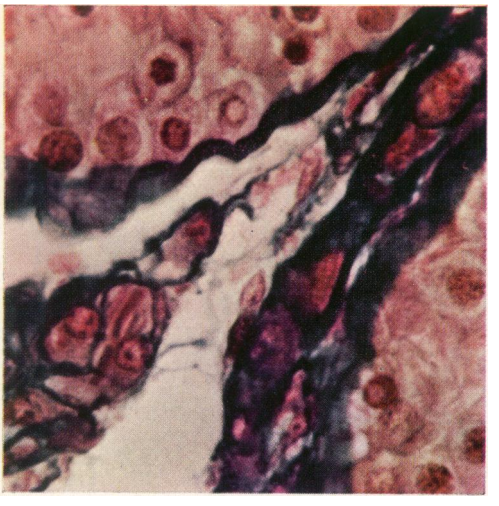

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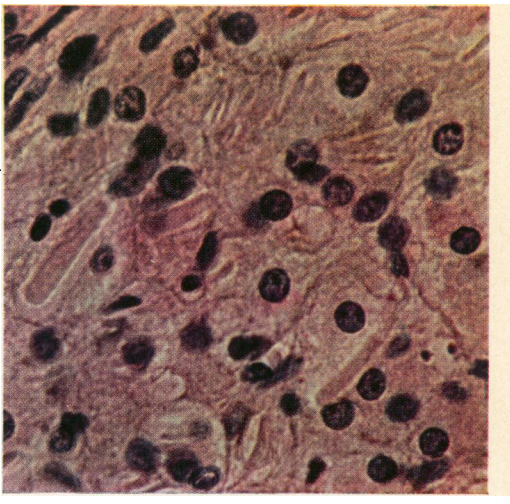

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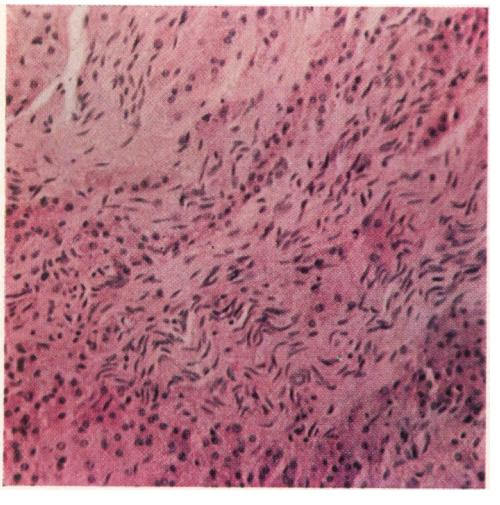

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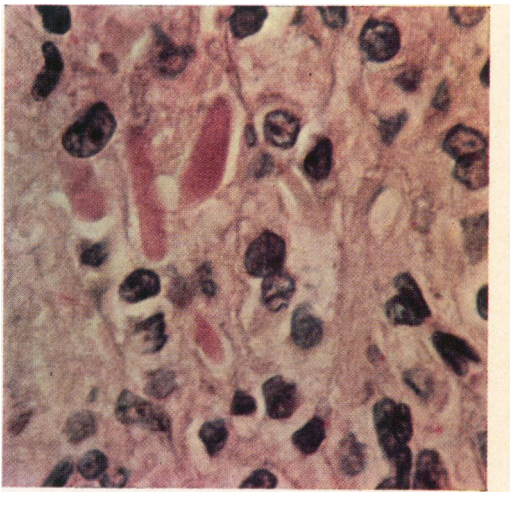

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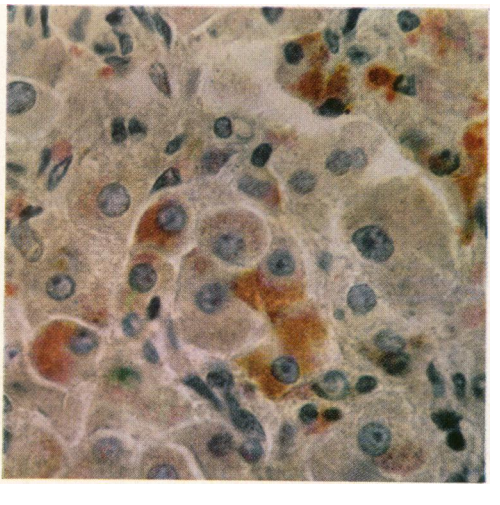

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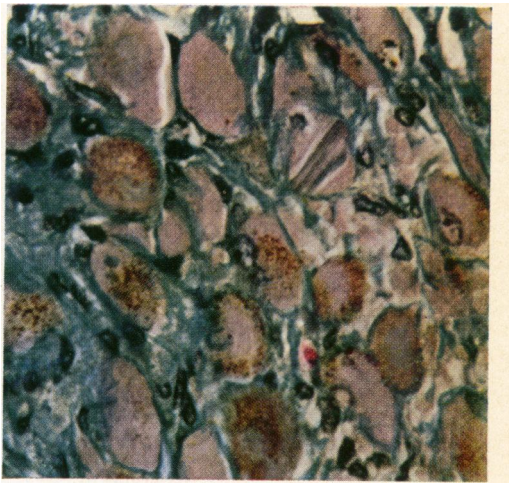

Sternberg

Ovarian Hilus Cells

PLATE 72

FIG. 7. Nest of hilus cells and associated myelinated nerves protruding into a dilated lymphatic space in the mesovarium of a normal woman, 30 years old. Hematoxylin and eosin stain. × 120.

FIG. 8. Protrusion of nonmyelinated nerve and hilus cells into a dilated lymphatic space, from the same case as Figure 7. Hilus cells lie between nerve fibers. Only a layer of endothelium separates the structure from the lumen of the lymphatic. Hematoxylin and eosin stain. × 250.

FIG. 9. Hilus cells, with a mitotic figure in the lower part of the field, from a patient who received 10,000 I.U. of chorionic gonadotropin for 19 days. The nonmyelinated nerve bundle, upper right, contains hilus cells between nerve fibers. Hematoxylin and eosin stain. × 490.

FIG. 10. Hilus cells and nonmyelinated nerve in another portion of the mesovarium from the same patient as Figure 9. The hilus cells show degenerative changes, with dark, acidophilic, nonvacuolated cytoplasm, loss of cell borders, nuclear pyknosis, and clumping of nuclei. Masson's trichrome stain. × 390.

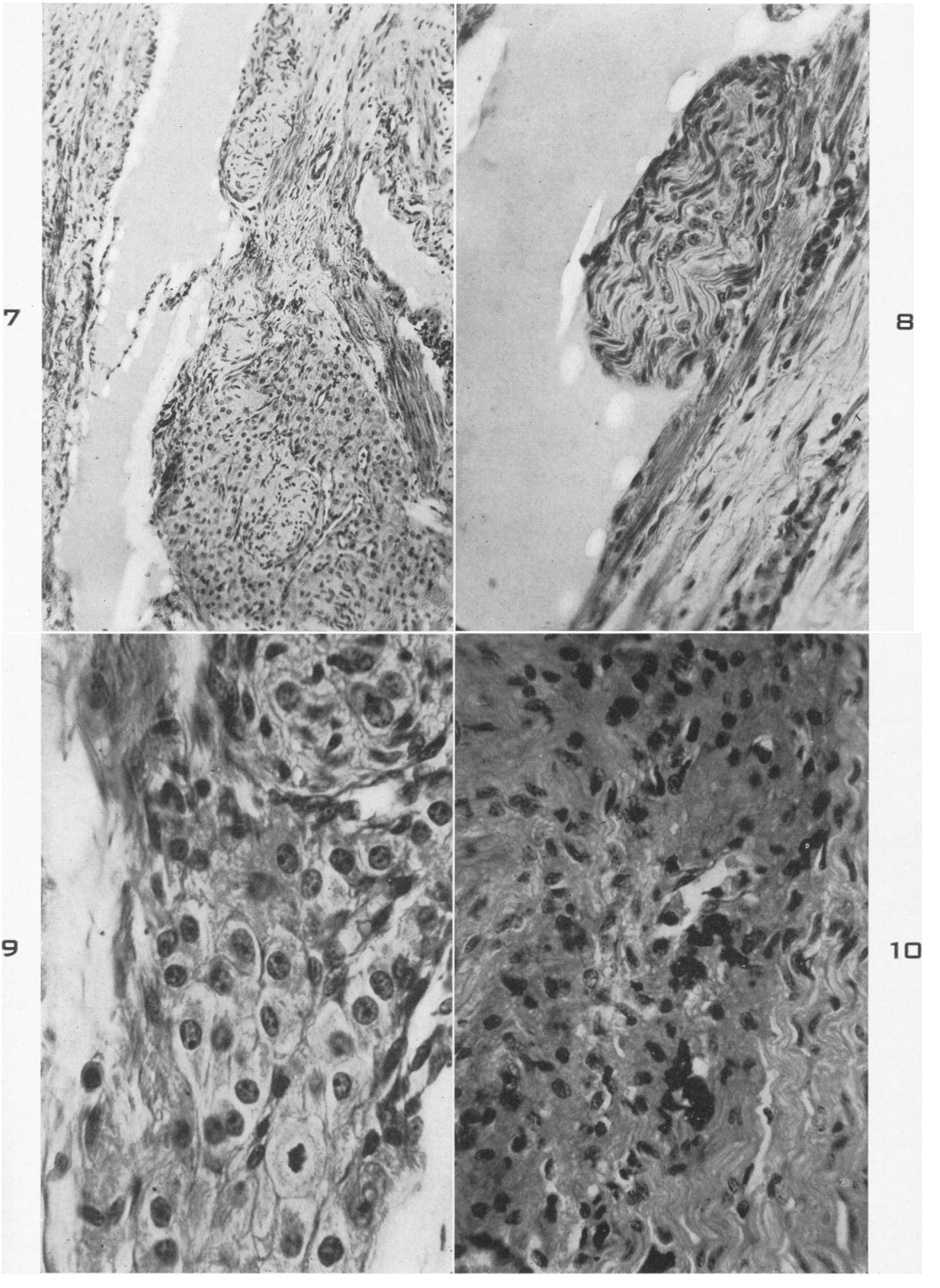
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PLATE 73

FIG. 11. Photograph of patient, case 1, an 86-year-old colored masculinized female with bilateral ovarian hilus cell tumors. Of note are the masculine features, mustache, and beard growth. (Published with the written permission of the patient.)

FIG. 12. Photograph of enlarged clitoris, case 1.

FIG. 13. Photograph of ovaries with ovarian hilus tumors, case 1. The ovaries have been split longitudinally toward the hilus, and laid open before photographing. The dark areas in the upper portion of the left ovary and in the lower portion of the right ovary are the tumors. They were located symmetrically at the medial pole of each ovarian hilus.

FIG. 14. Diagram of left ovary, case 1, showing relationship of the tumor, ovarian hilus, and adjacent structures. The area within the dotted line is plotted by projection from actual microscopic sections and represents the ovarian hilus and adjacent mesovarium. The large black area represents the hilus cell tumor. The smaller black islands represent hyperplastic nests of hilus cells. The ovarian ligament is to the right of the tumor.

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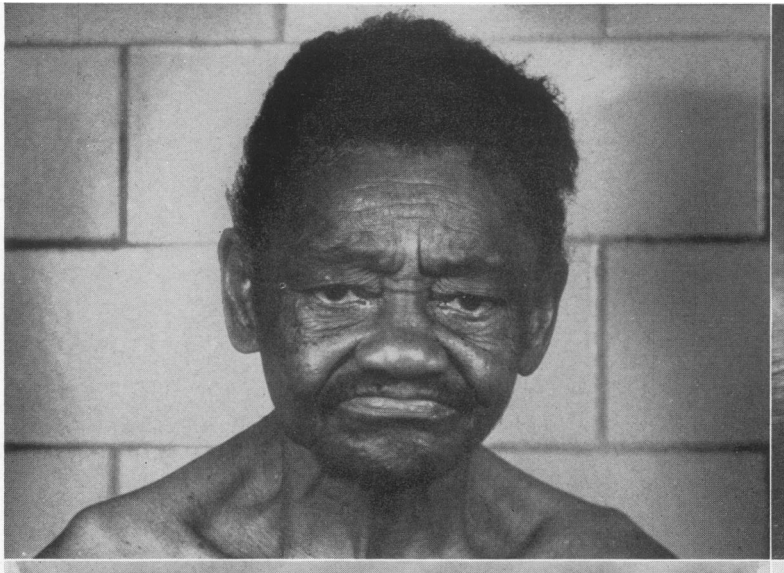

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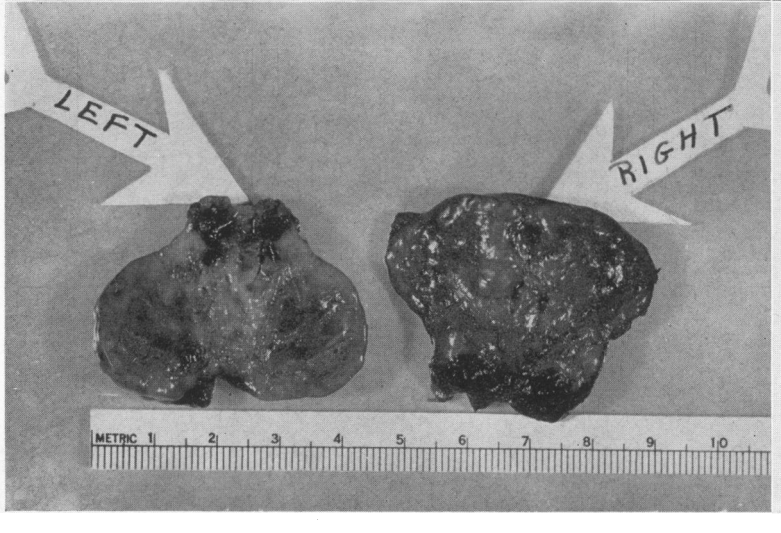

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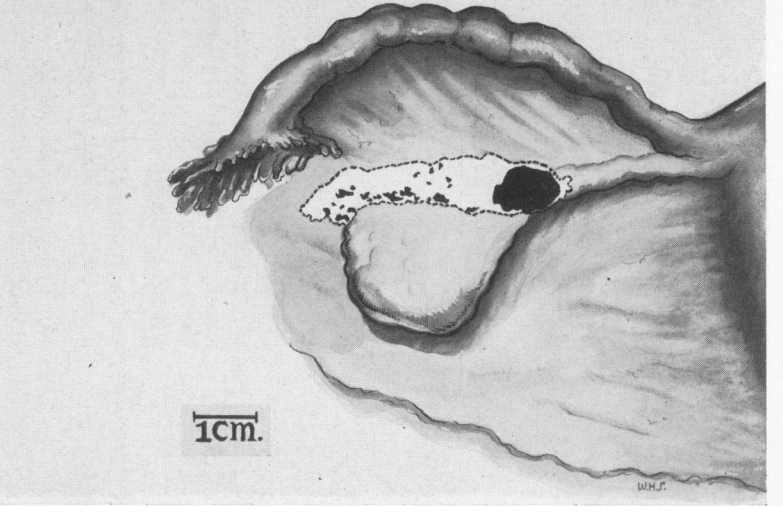


PLATE 74

FIG. 15. Ovarian hilus cells and nonmyelinated nerve at the periphery of the hilus cell tumor, case 1. Hematoxylin and eosin stain. × 160.

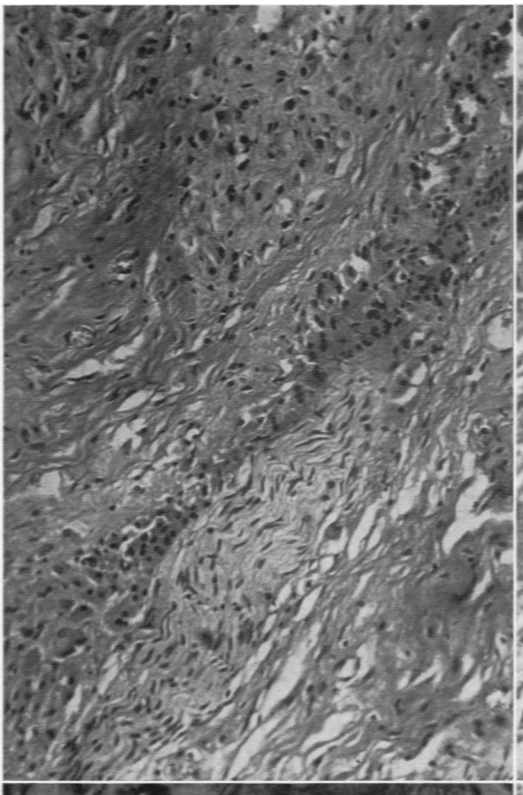
FIG. 16. Ovarian hilus cell tumor, case 1, showing elongated cell forms and greater morphologic variation than normal hilus cells. Crystalloid of Reinke is seen above and to the right of center. Two crystalloids are cut obliquely in the lower left field. Masson's trichrome stain. × 500.

FIG. 17. Crystalloid of Reinke from the hilus cell tumor, case 1. There is a clear space about the crystalloid. Phosphotungstic acid hematoxylin stain. × 800.

FIG. 18. Two crystalloids of Reinke from the hilus cell tumor, case 1. Delicate collagenous fibrils surround the cells. Phosphotungstic acid hematoxylin stain. × 650.

FIG. 19. Unusually large crystalloid of Reinke from the hilus cell tumor, case 1, with a surrounding clear space and longitudinal cleft. Phosphotungstic acid hematoxylin stain. × 800.

FIG. 20. Giant multinucleated hilus cells from the mesovarium of case 1, at some distance from the tumor. This is an occasional normal variation among hilus cells. Testicular Leydig cells may show similar forms. Hematoxylin and eosin stain. × 480.



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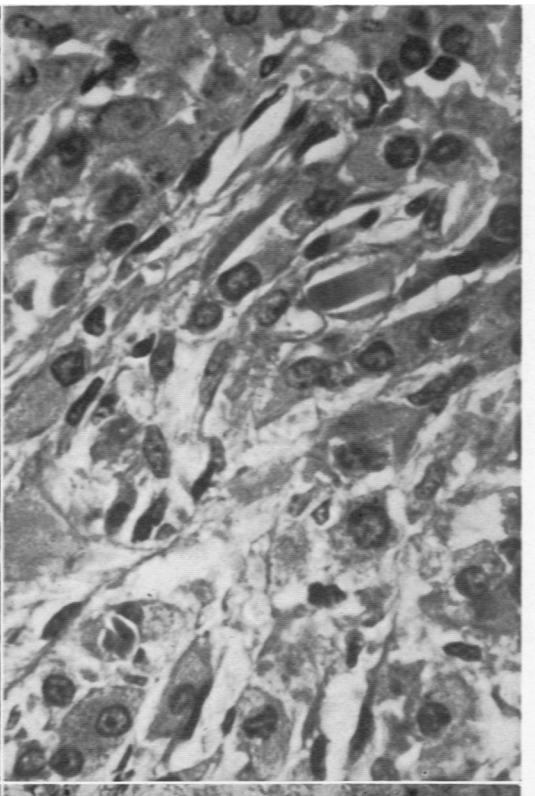

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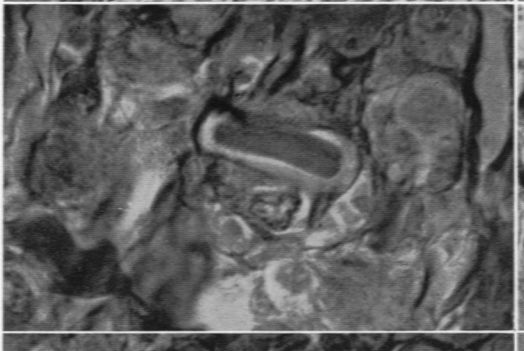

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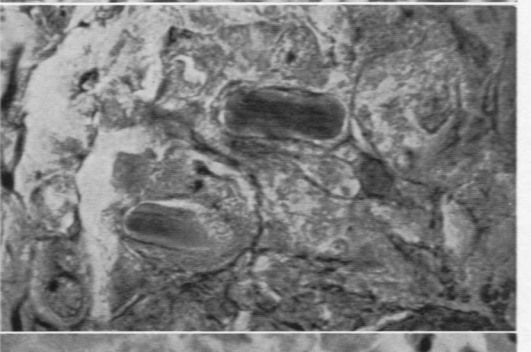

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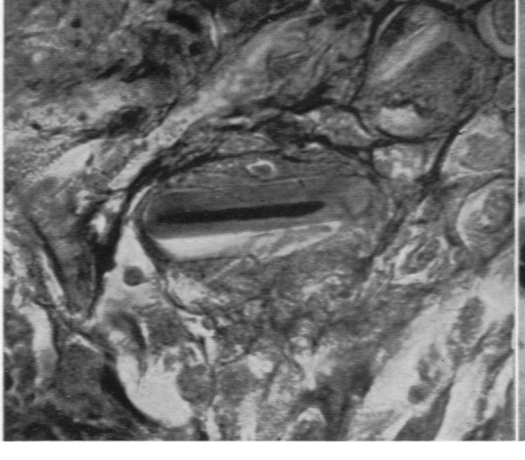

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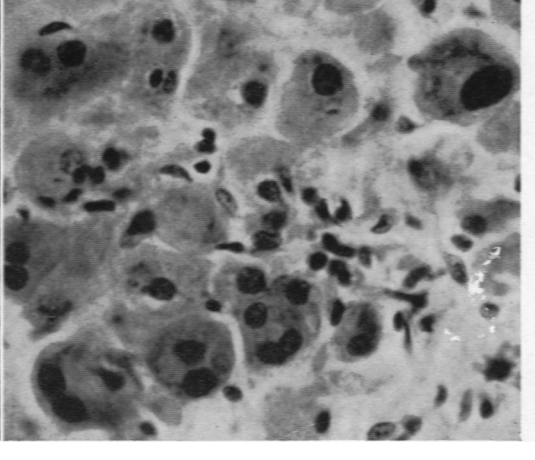

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PLATE 75

FIG. 21. Ovarian hilus cell tumor from case 2, a masculinized white female, 64 years old. The cells are very similar to normal hilus cells. Hematoxylin and eosin stain. × 160.

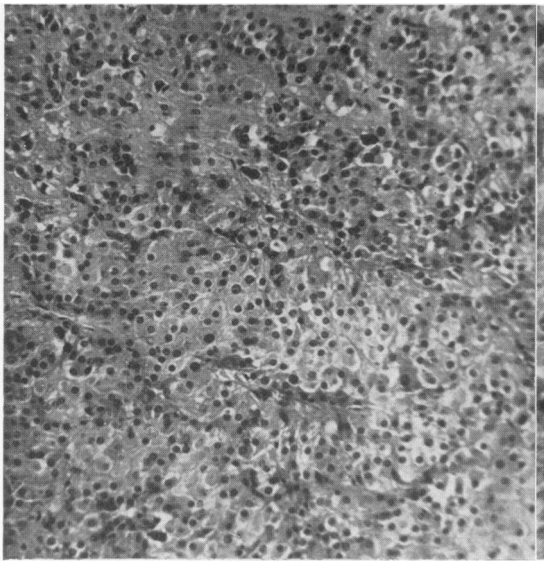
FIG. 22. Ovarian hilus cell tumor from case 2. Of note is the granular cytoplasm. Hematoxylin and eosin stain. × 670.

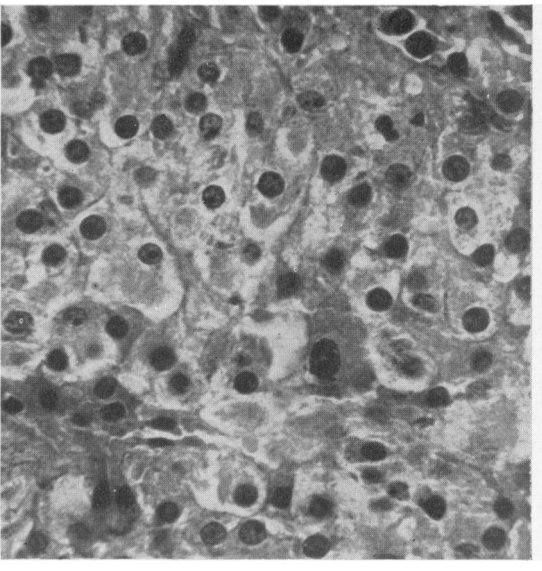
FIG. 23. Camera lucida drawing of tumor cells of case 2, showing "transition" of intracytoplasmic hyaline spherical bodies to crystalloids. Cells 1 and 2 (counting from upper left) contain ovoid and spherical bodies; cells 3 and 4 contain "transition" forms; cells 5 and 6 contain well developed crystalloids of Reinke.

FIG. 24. Photograph of masculinized 64-year-old white female, case 2, showing masculine features, recession of hairline, and mustache and beard growth. (Published with the written permission of the patient.)

FIG. 25. Typical adrenal rest in an ovarian hilus, an incidental finding in a routine surgical specimen from a patient 41 years old. Of note are the capsule, zone formation, organoid structure and simulation of normal cortex. For comparison with hilus cell nests. Hematoxylin and eosin stain. × 120.

21





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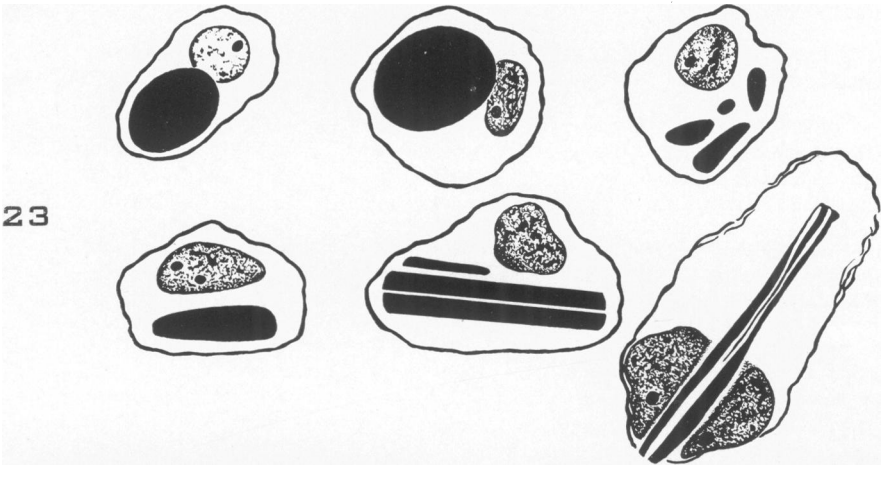

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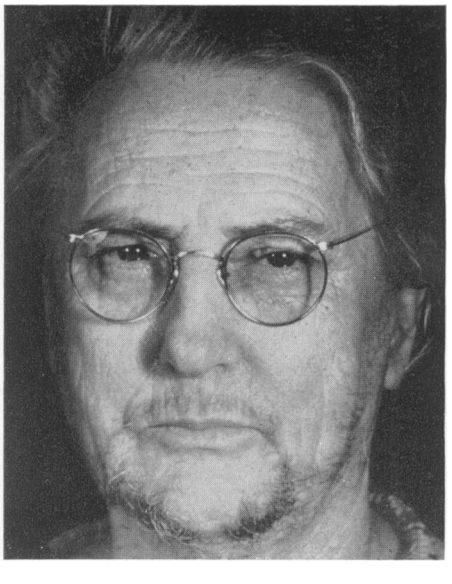
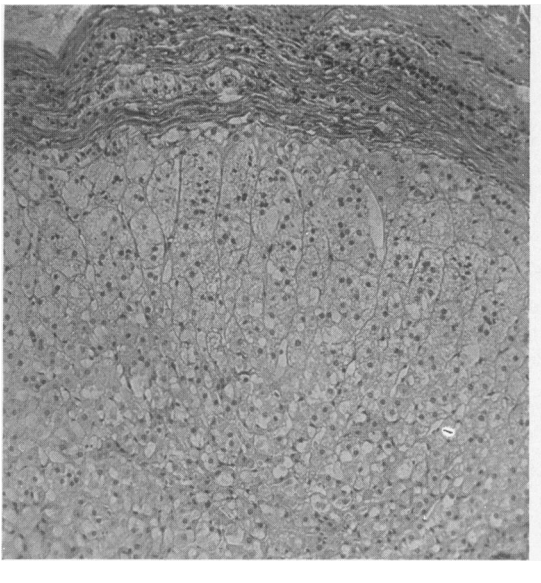


Sternberg



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Ovarian Hilus Cells