“SERTOLI” AND “LEYDIG” CELLS IN RELATION TO OVARIAN TUMOURS

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The group of tumours termed arrhenoblastomas was separated from the general body of ovarian tumours by Meyer (1915, 1930, 1931). Barzilai (1949) defines the arrhenoblastoma, in conformity with Meyer’s views, as “an ovarian tumour the cytoarchitecture of which almost exactly duplicates that seen in the different stages of male gonadogenesis.” Thus, although many of these tumours have a masculinizing or defeminizing effect on the host this is not essential for the diagnosis. Descriptions of about 122 of these tumours have been published (Javert and Finn, 1951). Three types of tumour pattern are recognized: (1) a highly differentiated pattern composed of well-developed tubules lined by cubical or columnar cells with basal nuclei, closely resembling Sertoli cells and the late developmental stages of gonadogenesis, as seen in the undescended testis; (2) one in which the tumour looks like a fibromatous or sarcomatous neoplasm; and (3) a pattern intermediate between these two extremes. Barzilai (1949) and Novak (1952) have drawn attention to the large polyhedral eosinophilic cells which frequently lie in the stroma of the tumours and resemble Leydig cells. The work of Sternberg (1949) and of Teilum (1949) allows the separation from this heterogeneous group of two distinct entities (a) “Leydig” cell tumours and (b) “Sertoli” cell tumours; a third form of tumour also exists in which both “Sertoli” cells and “Leydig” cells occur.

In this paper it is proposed to describe and discuss two tumours, which are morphologically arrhenoblastomas, in the light of these newer concepts, and also a case of bilateral Leydig cell tumour, or hyperplasia, with masculinization.

Case Reports

Case 1.—E. J., a married woman of 64, had menstruated irregularly from the age of 17 until her menopause at 50 years. There had been no pregnancies. For the last 14 years there had been facial hirsuties, increasing recently, and on admission she had a beard on the chin and lips. The pubic and axillary hair were feminine. Her voice had become masculine in the last 12 years. Her breasts were normal apart from a rubbery swelling \( \frac{1}{2} \) in. in diameter in one of them. She was obese. Her blood pressure was 200/130 mm. Hg. The vulva and vagina were atrophic. A radiograph of the sella turcica suggested no significant abnormality. On rectal examination a pelvic tumour was felt on the left side. The excretion of ketosteroids was 6.9 mg./24 hrs. (normal 8-17 mg./24 hrs.), and more than 19 mouse units of gonadotrophin were excreted in 24 hours (normal 12-25 mouse units/24 hrs., see Table I). The uterus and adnexae were removed. The patient was seen again 15 months later. There was no significant change clinically, the facial hirsuties persisting and obesity increasing, and the daily excretion of ketosteroids and gonadotrophins remained substantially the same as before the operation.

Macroscopic Appearances.—The uterus was lobular and enlarged by a number of fibroids. The ovaries were slightly enlarged, corresponding in size to those found in the child-bearing period; they contained small cysts and corpora albicantia.

Microscopic Appearances.—The ovaries are slightly enlarged and contain corpora albicantia and germinal inclusion cysts. The ovarian stroma is well developed and composed of intertwining bundles of connective tissue. In the interstices of the stroma are groups of cells with eosinophilic cytoplasm (Fig. 1). These are usually polygonal but in places the corners of the cells are rounded and in others drawn out giving a rather stellate appearance to the cells. The nuclei are round, each having a dense nuclear membrane, a well-defined but light chromatin pattern, and usually a nucleolus. These groups of cells are essentially similar to other groups present in unusually large numbers at the hilum of the ovary in close apposition to non-myelinated nerves and blood vessels, with the possible exception that the nuclei of the hilar cells are denser (Fig. 2).

The cytoplasm of the cells of both groups presents certain features: (1) Some cells possess brown pigment granules (Fig. 3). (2) Some of the cells at the hilum contain small bodies resembling Reinke crystalloids (Fig. 4) as seen in the interstitial cells of...
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17-Ketosteroids

Gonadotrophins (F.S.H.)

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Range

Case 1

Before

Operation

15 Months

after Operation

Case 2

Before

Operation

10 Days

after Operation

2 Months

after Operation

8-17

12-25∗

6-9 mg.

More than

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17-4 mg.

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24 hrs., and the gonadotrophin less than 8 mouse units

per 24 hrs. Two months later the corresponding figures

were 19.1 and 5 respectively (Table I).

Macroscopic Appearance of the Tumour.—This was

a yellow mass (6 x 4 x 1 cm.) with central cystic cavities.
The surrounding solid areas were lobular and flecked with yellow.

Microscopic Description.—The tumour has no well-

marked pattern but consists of sheets of fairly large cells showing a slight tendency to aggregate into clumps, the intervening connective tissue appearing to be oedematous. Small areas of haemorrhage are also present and a few large sinuses—possibly lymphatics. The main cells of the tumour have fairly large nuclei each having a well-marked nuclear membrane and a light chromatin pattern in which frequently a nucleolus can be seen. The cytoplasm of these cells is faintly eosinophilic and the cell margins are ill-defined. In one place these cells are arranged in a radial manner giving rise to acinar structures (Fig. 5). Scattered through the tumour, singly or in groups, are cells with markedly eosinophilic cytoplasm (Fig. 6). They are usually polygonal or rounded, but occasionally elongated, and have well-defined cell boundaries. The nuclei of these cells are similar to those of the main cells of the tumour but tend to be larger each having a nucleolus, and sometimes more than one nucleolus. These cells present the following features: (1) Most, probably all, of these cells give a positive reaction with Vines stain. (2) A very few cells give a positive P.A.S. reaction. (3) The cytoplasm is finely vacuolated. Some of the larger of these cells have taken on a xanthomatous appearance. Much fat is present in the cytoplasm of these eosinophilic cells but one cannot be sure that it is confined to these cells only. (4) Many of the eosinophilic cells, possibly all, stain darkly with phosphotungstic acid haematoxylin. (5) No brown pigment or Reinke crystalloids are present (cf. Leydig cells of premature testis).

Reticulin fibres sometimes surround these groups of eosinophilic cells and at others penetrate the groups separating the individual cells. A comparison of the eosinophilic cells in these two cases with ovarian hilar cells and testicular Leydig cells is shown in Table II. It can be seen that the eosinophilic cells of the ovarian lesions are very similar, their differences being of the same type and order as the differ-
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Fig. 1.—Case 1: A group of "Leydig" cells in the stroma of the ovary (Vines stain \( \times 400 \)).

Fig. 2.—Case 1: A group of "Leydig" cells at the ovarian hilum. Note the nerve below and the blood vessel above (haematoxylin and eosin \( \times 400 \)).

Fig. 3.—Case 1: Pigment granules in the eosinophilic cells of the ovarian stroma (haematoxylin and eosin \( \times 1,000 \)).

Fig. 4.—Case 1: A group of "Leydig" cells from the ovarian hilum. The cell above and to the left of the centre of the field contains a long, fairly broad, rod-shaped crystallloid of Reink which crosses in front of the nucleus and occupies much of the cell (Masson's trichrome stain \( \times 500 \)).
Fig. 5.—Case 2: A group of the main cells of the tumour forming an acinus (haematoxylin and eosin ×400).

Fig. 6.—Case 2: The margins of the main cells of the tumour are ill-defined and the nuclei are irregularly arranged. The eosinophilic cells form groups having an orderly arrangement. The cytoplasm of these cells is finely vacuolated (haematoxylin and eosin ×400).

Fig. 7.—Case 3: The boundaries of the cells cannot be defined and the cytoplasmic tendrils intermingle in the lumen. There is a tendency for the acini to be lined by more than one layer of cells (haematoxylin and eosin ×400).

Fig. 8.—Case 3: The dark cells are eosinophilic "Leydig" cells in the band of tissue connecting the left ovary to the broad ligament. Note the close relationship of these cells to a blood vessel (Vines stain ×400).
Acidophil + Vacuolation (Sudan Phosphotungstic acid...)

On examination of the ovary on October 7, 2023 by guest. Protected by copyright. Downloaded from http://jcp.bmj.com/ J Clin Pathol: first published as 10.1136/jcp.7.1.10 on 1 February 1954. Copyright © 1954 BMJ Publishing Group Limited. All rights reserved. Protected by copyright. Downloaded from http://jcp.bmj.com/ J Clin Pathol: first published as 10.1136/jcp.7.1.10 on 1 February 1954. Copyright © 1954 BMJ Publishing Group Limited. All rights reserved.
the cytoplasm of intra-ovarian cells distinguishes them from lutein cells. The normal level of excretion of 17-ketosteroids renders the origin of the "Leydig" cells from ectopic adrenal tissue unlikely.

It may be that Case I should more properly be termed one of hyperplasia rather than of a neoplasm. In favour of the condition being hyperplastic is its bilateral situation, but against this there is the apparent invasion of the ovarian stroma by hilus cells unless it is supposed that the intra-ovarian Leydig cells arise by metamorphosis of the stroma. In the two cases of hilus-cell hyperplasia with masculinization reported by Sternberg (1949) the cells were confined to the hilum.

Case I bears some resemblance to the cases of Geist and Gaines (1942) and Rottino and McGrath (1943) in which luteinization of the ovaries is described in association with masculinization. The criteria used by these workers in determining the lutein nature of the ovarian change are uncertain and hormone analyses are lacking.

The general architecture of the tumour in Case 2 is of an arrhenoblastoma of Meyer's intermediate class. The eosinophilic cells resemble the Leydig cells of the foetal testis and also the hilar cells of the infantile ovary, but lack the pigment and crystalloids of the adult cells. Berger (1942) reported a virilizing tumour of the mesovarium in a woman of 50. This he considered to be of "Leydig" cell type although Reinke crystalloids and cytoplasmic pigment were not present. Although lutein cells have many of the features of hilar cells (including a positive Vines reaction) only a trace of pregnanediol was excreted, thus rendering it unlikely that the eosinophilic cells of this tumour were lutein. A positive Vines reaction is also given by cells in the foetal adrenal cortex, in hyperplastic adrenal cortex and in cortical adrenal tumours (Broster and Vines, 1933; Zinsser and Zinsser, 1951). Thus the eosinophilic cells of this tumour might arise from ectopic adrenal tissue near, or in, the ovary, but in adrenal hyperplasia and masculinizing adrenal tumours the excretion of 17-ketosteroids is usually greatly increased. Normal excretion can occur, although very rarely (Escamilla, 1949; Kepler and Mason, 1947; Venning and Browne, 1947). The excretion of 17-ketosteroids in the two patients of Sternberg (1949) with hilar cell tumours was normal; in the patient of Waugh, Venning, and McEachern (1949) it was moderately increased but the left adrenal was also enlarged. The patient of Sachs and Spiro (1951) had a normal 17-ketosteroid excretion but this may have been due to coincidental malignant cachexia. All these tumours were identifiable as "Leydig" cell tumours by their site and by the presence of Reinke crystalloids in the cytoplasm of the constituent cells. Thus in morphological features and on the grounds of hormone excretion the eosinophilic cells of Case 2 are compatible with a "Leydig" cell origin and are not lutein or adrenal in nature.

Sternberg (1949) considered that his two cases of hilus-cell tumour, and that of Berger (1942) and another mentioned in an addendum to his paper, to be the only hilus-cell tumours reported up to then. The above analysis suggests that his view is too limited and that eosinophilic cells seen in many arrhenoblastomas and described as Leydig cells (Brentnall, 1945; Teilm, 1949) without further specification of their properties are, in fact, "Leydig" (or hilus) cells. Besides arrhenoblastomas, other groups of masculinizing ovarian tumours occur associated with eosinophilic cells; these have been termed "virilizing lipoïd cell tumours" (Barzilai, 1949) and "adrenocortical tumours" (Herbut, 1953). It seems probable that some tumours such as that of Merivale and Forman (1951), which had a high urinary output of 17-ketosteroids, are adrenal rather than "Leydig" in type.

The Nature of the Tubular Adenomas.—The characteristic features of the epithelium lining the tubules in Case 3 are (i) the palisade arrangement of the cells, (ii) the vacuolated fibrillar cytoplasm, (iii) the ill-defined cell boundaries with interlacing cell processes (see Fig. 7), giving a syncytial appearance, and (iv) the presence of sudanophilic granules. These features are found in the Sertoli cells in atrophic testes (Innes, 1942; Stalker and Hendry, 1952).

Pick (1905) recognized the morphological similarity between these tumours and testicular tissue, but this similarity may also extend to their function. Pick's patient was a woman of 34 suffering from metrorrhagia and had no masculinizing signs. Likewise the patients described by Schickele (1907), Grevle (1936), Henderson (1942), and Dougal (1945) had menorrhagia or metrorrhagia. In the case recorded by Burslem, Langley, and Woodcock (1953) the patient had irregular vaginal bleeding associated with cystic hyperplasia of the endometrium. These features suggest that such tumours are oestrogenic rather than androgenic. Similar tumours occur in the testis and, although they usually have no hormonal effect (Stalker and Hendry, 1952), Teilm (1946) has described such a tumour in a man of 53 years associated with gynaecomastia and increased
oestrogen excretion. Likewise feminizing testicular tumours of similar morphology have been described in dogs by Innes (1942) and by Huggins and Moulder (1945).

Although these tubular (or Sertoli cell) tumours of the ovary are often feminizing they are occasionally masculinizing, as in Meyer's (1930) Case 2 and in Phelan's (1934) patient. Sometimes they are hormonally indifferent and such tumours are often associated with rudimentary sex organs or their congenital absence (Novak, 1943; Jolles and Gleave, 1945; Goldberg and Maxwell, 1947). My Case 3 falls into this group. It is interesting to note that of the 17 cases of tubular adenoma of the testis which Stalker and Hendry (1952) have collected from the literature, 11 occurred in pseudohermaphrodites.

The Histogenesis of Sertoli Cell Tumours.—Whereas "Leydig" cells appear to be a very frequent, if not constant, component of the ovary Sertoli cells are normally confined to the testis. It must however be remembered that, whatever the details of embryogenesis, Sertoli and granulosa cells have the same Anlage (Gillman, 1948) and cannot be distinguished until about the 15 mm. stage in the human foetus (Norris, 1938). Thus it is evidently possible under abnormal conditions for the precursor of the definitive granulosa cell to mature along an alternative line and give rise to a Sertoli cell. That this can happen is shown by the case of Burslem et al. (1953) in which both a Sertoli cell tumour and a granulosa cell tumour merge. Meyer (1930) described two tumours (Cases 4 and 7) showing a similar merging.

Interference with differentiation may cause an apparent reversal of sex (Popoff, 1930; Berger, 1945). Danitchakoff (1936) has produced the full feminization of genetically male birds by introducing oestrone dissolved in oil into the allantoic cavity on the fourth day of incubation. After hatching these developed as intersexes. Danitchakoff (1937) has also produced masculinization of guinea-pig embryos by use of testosterone propionate. Lillie (1916, 1917) showed that when the foetal membranes of male and female calf embryos become united in utero so that the circulations are in continuity the female partner becomes greatly modified in the male direction forming an intersex (freemartin) with testes and male ducts. Remembering that many of these Sertoli cell tumours both in men and women are associated with congenital anomalies of the genital tract the possibility that the tumour itself is a result of disturbed gonadogenesis becomes likely, the disturbing factor possibly being in the maternal hormone system. Thus Sertoli cell tumours of this class should be considered as congenital malformations, or hamartomas, rather than blastomas. This concept may, perhaps, apply to other Sertoli cell tumours in which disturbance of development has not involved the rest of the genital tract.

Conclusion

Accepting the concept of the term arrhenoblastoma as introduced by Meyer and crystallized in the definition of Barzilai (1949) as "an ovarian tumour the cytoarchitecture of which almost exactly duplicates that seen in the different stages of male gonadogenesis," it is possible to subdivide them into at least three groups: (a) those in which "Leydig" cells are a conspicuous feature, (b) those in which "Sertoli" cells are conspicuous, and (c) a mixed group in which both "Sertoli" and "Leydig" cells occur. Such a classification does not include the virilizing lipid-cell tumours, nor the masculinizing tumours with a "sarcomatous" pattern.

Summary

Two patients with masculinization of ovarian origin are described. The first had bilateral tumours, or hyperplasia, of ovarian hilus (extratubular Leydig) cells and the second had an arrhenoblastoma in which "Leydig" cells were a conspicuous feature.

A third patient is described who had bilateral ovarian tumours of tubular, "Sertoli" cell, type associated with congenital malformation of the genital tract.

The relationship of these tumours to the classification and histogenesis of arrhenoblastomas is discussed.

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REFERENCES


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