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Case Report

Massive Ovarian Oedema with Production of Testosterone

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Summary. Clinical, biochemical, light- and electron microscopic, and immunocytochemical findings of a $13^1/_2$ year old girl with delayed menarche and signs of virilization due to massive oedema of the left ovary with activation of stromal cells (hyperthecosis) are presented. Testosterone and oestradiol production by large cells in the voluminous ovary was demonstrated by immunocytochemistry and radioimmunoassay. Massive ovarian oedema may result from partial or intermittent torsion of the mesovary interfering with venous and lymphatic drainage, but not with arterial blood flow.

Key words: Ovarian oedema – virilization – testosterone.

Introduction

Massive oedema of the ovary simulating fibroma was first described as a distinct clinico-pathologic entity by Kalstone et al. (1969). We report clinical, biochemical, light- and electron microscopic, and immunocytochemical findings in a patient suffering from stromal oedema of the ovary who presented with signs of virilization.

Clinical Data

A $13^1/_2$ year old girl (bone age: 13 years) was investigated because of the complaint of absence of puberty, amenorrhea and a somewhat husky voice. Physical examination revealed a tall girl (172.5 cm, 97 P; body weight 59 kg, 25 P) with a discrete hirsutism of the legs and a hypertrophy of the clitoris which measured 2 cm. The development of the breasts was in stage 2+, that of pubic hair in stage 3+ according to Tanner (1962).

Protrusion of the abdomen, due to a large palpable mass was noted. At intravenous pyelography and sonotomography the mass was localised in the anterior part of the lower right abdomen.

Plasma *testosterone* was elevated to 11.1 and 15.6 nmol/l, a normal adult male level (normal range 0.7–4.2). 17-α-hydroxyprogesterone was within normal limits (8.5 nmol/l), thus excluding

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a classical 21- or 11-β-hydroxlase deficiency. The plasma level of *oestradiol* was elevated to 242 pmol/l which corresponds to the ovulation peak-value in adult women.

Urinary excretion of *dehydroepiandrosterone* (DHEA) was elevated to 9.0 µmol/24 h (normal range 0.17–5.2). Excretion of 17-hydroxy steroids (18.8 µmol/24 h) and 17-ketosteroids (17.3 µmol/24 h) was in the normal range.

A testerone producing tumor was suspected. At *laparotomy* a white, firm mass (1,900 g, 24 × 14 × 8 cm) of the left ovary was found. Its pedicle was partially twisted. The mass, the left Fallopian tube and two paraaortic lymph nodes were excised. In addition a wedge-resection of the moderately enlarged right ovary was performed.

Postoperatively the hirsutism disappeared but the husky voice and the hypertrophy of the clitoris persisted. On the eighth postoperative day plasma testosterone was normalised (0.7 nmol/l) and plasma oestradiol fell to 51 pmol/l corresponding to the bone age and the clinical pubertal development of the patient.

Morphologic and Biochemical Investigations

Tissue samples from the surgical specimen were immersed in buffered liquid formaldehyde (4%) and embedded in paraffin. Deparaffinized sections (5 µm) were stained with H+E, van Gieson's stain, Novotny's stain for reticulin fibres and Masson's trichrome.

Other specimens were immediately fixed in glutaraldehyde (3%), in phosphate-buffered saline (PBS, pH 7.2), postfixed in osmium tetroxide (1%) in PBS (pH 7.2), dehydrated in graded ethanol and embedded in Epon. Semithin sections (1 µm) were stained with toluidine blue. Thin sections (600–800 nm) were collected on copper grids, stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 electron microscope.

Immunocytochemistry was carried out on deparaffinized sections (5 µm). Testosterone was localized using the unlabeled antibody enzyme method (Sternberger 1979). The antiserum[1] was raised in rabbits using a testosterone-3-bovine serum-albumine conjugate. The antiserum crossreacts to 77% with dihydro testosterone. Other crossreactions are negligible (androstendion 0.8%, androsterone 0.02%, DHEA 0.02%, etiocholanolone 0.004%).

The antiserum was diluted with PBS (pH 7.2) from 1/1,000 to 1/8,000 and was absorbed with bovine serum albumin (1 µmol). All sera were decomplemented prior to use. The histochemical reaction for peroxidase was carried out using 3,3′-diaminobenzidine-tetrahydrochloride (DAB; 0.05% w/v) and hydrogen peroxide (0.01%) in 0.05 M Tris-HCl buffer (pH 7.6). After fixation with osmium tetroxide (1%) in PBS the sections were dehydrated and mounted.

Controls

Normal testicular tissue fixed in liquid formaldehyde or Stieve's fluid, the testes of a patient suffering from testicular feminization (with secretion of large amounts of testerone due to hyperplasia of interstital cells), normal adrenal glands and ovaries fixed in liquid formaldehyde were used as control tissues.

Control reactions were carried out as follows:

a) Anti-testosterone serum preabsorbed with 1 nmol of testosterone (Serva), β-oestradiol (Serva), or progesterone (Serva) as first layer.

b) PBS and non-immune sera as first, second or third layers.

c) Omission of DAB or hydrogen peroxide from the incubating medium for the peroxidase reaction.

Biochemical investigations were carried out on tissue samples which were immediately deep-frozen. The tissue was homogenized in PBS and subsequently extracted with ether. The content of testosterone and estradiol was determined in a plasma radioimmunoassay run.

Results

The "tumor" was surrounded by a white, translucent capsule. The cut surface was white, glistening and moist, and contained many cystic spaces from which a proteinaceous fluid discharged (Fig. 1).

1 The anti-testosterone antiserum was a gift of Dr. A.A.A. Ismail, Edinburgh

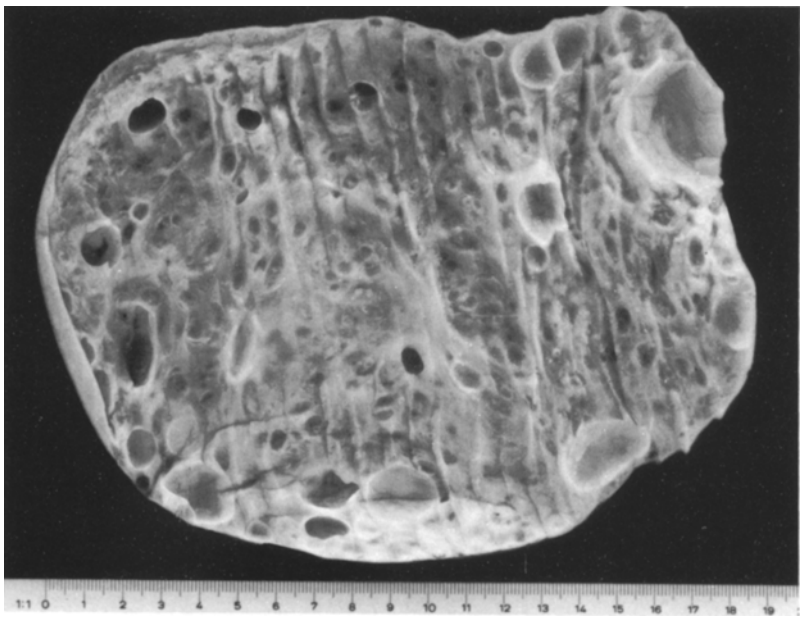


Fig. 1. Cut surface of ovary with a large number of cystic spaces

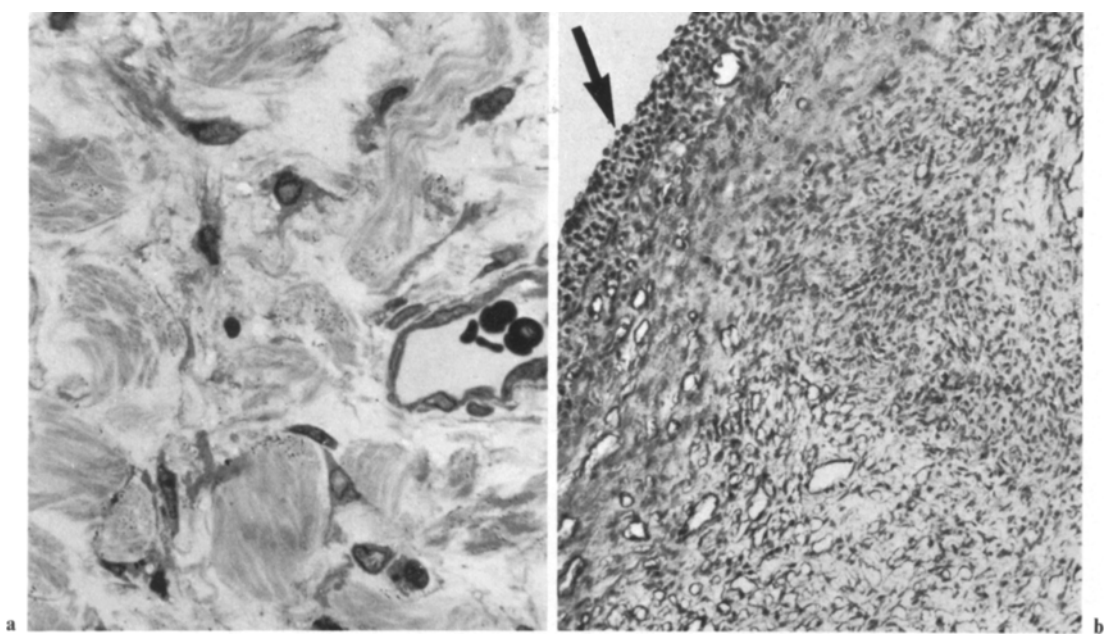
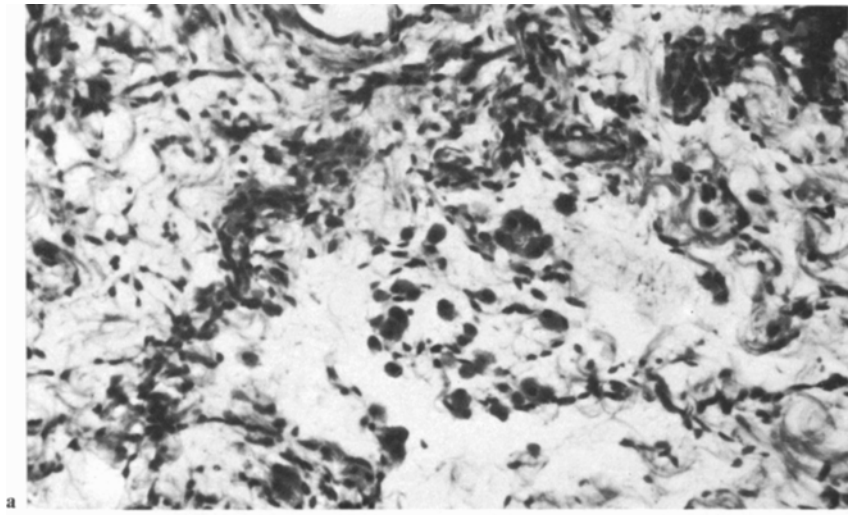
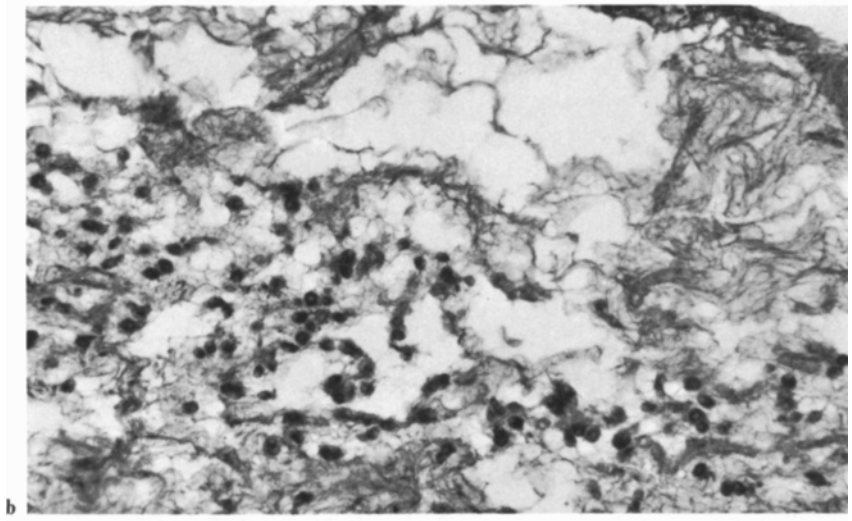


Fig. 2. a Stromal cells with long processes separated by oedematous fluid and bundles of collagen fibers. At right a dilated blood capillary. Semithin section, Toluidine blue, × 533. **b** Part of a follicle (arrow) surrounded by a band of dense fibrous stroma. Massive oedema of the other stromal areas, in which a large number of dilated vessels are present. H+E, × 133



a



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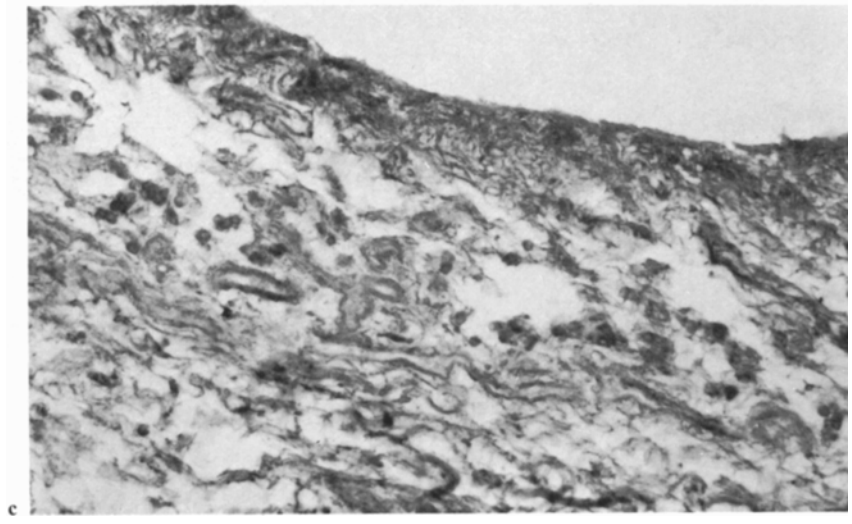

c

At *light microscopy* marked subcapsular fibrosis was noted. The ovarian stroma was severely oedematous, the accumulation of faintly eosinophilic fluid being most pronounced in the inner cortex and in the medulla. Bundles of collagen fibers were widely separated by the fluid (Fig. 2a). Markedly dilated blood vessels were numerous. A small number of large atretic follicles with a loose network of collagen fibers and fibroblasts were scattered throughout the oedematous zone. The tissue surrounding atretic follicles as well as primary and secondary follicles was dense and highly cellular (Fig. 2b). It contained clusters of large cells with a dense nucleus and an eosinophilic cytoplasm resembling luteinized cells (hyperthecosis; Fig. 3a). The cells contained a lipochrome pigment. There were no areas of necrosis. No mitotic figures could be detected in the stroma.

The opposite ovary contained multiple follicular cysts and a moderate subcapsular fibrosis, but no oedema was noted.

At *electron microscopy* individual stromal cells were widely separated by collagen fibers and an amorphous electron-lucent material. Fibroblasts and fibrocytes were surrounded by a narrow band of collagen fibers. Their nucleus was elongated and contained prominent marginated heterochromatin. A nuclear fibrous lamina (thickness about 60 nm) adjacent to the inner membrane of the nuclear envelope was conspicuous. In the cytoplasma abundant bundles of fibrils, areas of free ribosomes, prominent Golgi complexes and exocytotic vesicles at the cellular membrane were seen. Some other cells contained large lipid droplets. No cells suggestive of steroid production could be identified with certainty.

At *immunocytochemistry* the large cells located mainly at the periphery of the atretic follicles (probably theca cells) were found to contain cytoplasmic testosterone-immunoreactivity. No other cells reactive to the anti-testosterone antibodies were seen (Fig. 3b).

Controls. A strongly positive cytoplasmic reaction was observed in the interstital cells of normal testicles and of the testes of the patient suffering from testicular feminization, whereas no reaction product was present in the normal adrenal cortex and ovaries. *Absorption* of the anti-testosterone serum with testosterone prior to incubation completely abolished the immunocytochemical reaction (Fig. 3c), while a strongly positive reaction persisted upon incubation with anti-testosterone serum preabsorbed with β-oestradiol and progesterone. All other control reactions were invariably negative.

In the *radioimmunoassay* of the tissue extract 6.5 ng of testosterone and 67 ng/g wet tissue of oestradiol were found.

Fig. 3. a Luteinized stromal cells with eosinophilic cytoplasm. H+E, ×133. **b** Cytoplasmic testosterone-immunoreactivity in a group of large cells. Anti-testosterone antibody diluted 1/8,000, ×133 **c** Lack of testosterone-immunoreactivity after absorption of anti-testosterone antibody (diluted 1/8,000) with 1 nmol of testosterone prior to incubation. The patchy "staining" of the cytoplasm is due to the presence of lipochrome pigment. ×133. **b, c** Unlabeled antibody enzyme method

Discussion

Massive oedema of the ovary is apparently uncommon. To our knowledge only 18 patients have been described so far (Kalstone 1969; Zourlas and Jones 1969; Roth 1971; Bezahler 1974; Val Bernal 1974; Lupovitch and Shanoski 1974; Kim and Rozanski 1976; Nassar et al. 1976; Tegelbjaerg and Vetner 1977; Kindermann and Christ 1977; Roth et al. 1979). All patients were less than 33 years of age (range $6^1/_2$–33), two patients were premenarchal. All but one patient suffered from unilateral ovarian oedema.

Thirteen patients presented with an abdominal mass, sometimes described as cystic. Chief complaints were abdominal pain and irregular menses. In only 3 patients were signs of virilization present.

Elevated preoperative urinary excretion of 17-ketosteroids was found to return to normal after surgery in 1 patient (Kalstone et al. 1969). In a $6^1/_2$ year old girl with precocious puberty and elevated urinary 17-ketosteroids, clinical symptoms disappeared after surgery (Roth et al. 1979). Unfortunately, postoperative serum concentrations of sex steroids of this patient and of the 2 patients with preoperative virilization were not reported.

The surgically removed ovaries were all markedly enlarged (weight up to 2.4 kg), oedematous and contained many cysts. Torsion of the mesovary was observed in 8 patients. At light microscopy bundles of collagen fibers widely separated by an oedema fluid and the occurrence of primary and atretic follicles are characteristic. In 6 ovaries large cells with an eosinophilic cytoplasm, thus resembling luteinized steroid-producing cells were observed (Roth et al. 1979). One of these patients was virilized before surgery (Kalstone et al. 1969). In 3 ovaries a stromal hyperplasia was found (Roth et al. 1979).

Clinical symptoms of the patient described here and morphological observations on the surgically removed ovary fit with the description of the 18 patients previously reported. In addition, production of testosterone and oestradiol by the oedematous ovary was demonstrated by the following observations: 1) Before surgery the serum concentration of testosterone and urinary excretion of dehydroepiandrosterone were well in the range of a normal adult male, 2) Preoperative serum concentration of oestradiol in presence of normal levels of FSH and LH corresponded to a follicular phase of a normal adult female, 3) Serum levels of testosterone and oestradiol fell to values in the normal range after surgery, 4) Signs of virilization disappeared after excision of the oedematous ovary, 5) The production of testosterone by luteinized theca cells around atretic follicles could be shown by immunocytochemistry, and 6) Testosterone and oestradiol concentration in tissue extracts were markedly elevated when compared with normal ovaries.

The *pathogenesis* of massive ovarian oedema with hyperthecosis is not clear. However, dilated blood capillaries, veins and lymph vessels with marked stasis of blood and lymph, haemorrhagic areas suggesting developing infarction (Roth et al., 1979), and torsion of the mesovarium observed in 9/18 patients argue in favour of an incomplete, perhaps intermittent, torsion of the mesovary interfering with venous and lymphatic drainage but not with arterial blood flow. The source of increased testosterone and/or oestradiol production are activated

stromal cells as seen in hyperthecosis. They do not appear to be hormonally stimulated because the lesion is unilateral. We therefore assume that chronic hypoxia somehow induces a hyperthecosis with increased production of testosterone.

The *diagnosis* of ovarian oedema should be considered if a young woman presents with a large abdominal mass, which is localized to one ovary at laparotomy.

It can be established by examination of a frozen section. The differential diagnosis includes oedematous fibroma, unilateral thecoma (Longcope et al. 1980), and sclerosing stromal tumor (Chalvardjian and Scully 1973). In presence of these tumors part of the ovary is replaced by the tumor mass and non-tumorous ovarian parenchyma is distorted. By these criteria tumors can be differentiated from massive ovarian oedema which does not destroy ovarian parenchyma. If ovarian oedema is bilateral a wedge resection will cure the patient (Kindermann and Christ 1977).

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