CLINICAL CONFERENCE
Chromosomal Sex and the Prepuberal Diagnosis of Gonadal Dysgenesis

By Melvin M. Grumbach, M.D.
Department of Pediatrics, College of Physicians and Surgeons, Columbia University

Dr. GRUMBACH: Barr and associates have demonstrated that in the human the majority of somatic cells of females contain a conspicuous, heterochromatic mass of chromatin in the resting nuclei. Their discovery of a sex-difference in intermitotic nuclei of a number of vertebrate species, including man, has provided a relatively simple method for assessing the sex-chromosome constitution. This chromatin mass is about 1 micron in diameter and often plano-convex in configuration. It is usually located against the inner surface of the nuclear membrane and contains deoxyribonucleic acid. In males, a comparable chromatin mass is rarely found, never in more than a few per cent of the nuclei. There is good evidence that this so-called “sex-chromatin” represents the fusion of heterochromatic portions of two X-chromosomes.

The sex chromatin can be conveniently determined by examination of specimens of skin obtained by biopsy (Fig. 1). Recently, more practical methods for determining cytologic sex have been described employing smears from readily available tissues, such as the oral and vaginal mucosa (Fig. 2) and the blood. Davidson and Smith have shown that there is a sex difference in the morphology of polymorphonuclear neutrophils.

Cytologic examination of chromosomal sex has provided an important tool for the investigation of anomalies of sex development. Apart from its ancillary role in diagnosis, cytologic examination of sex chromatin has made a significant contribution to our understanding of the disordered development in these afflictions. However, the results of this determination should not be regarded as an especial indication of the psychosexual orientation of patients with such abnormalities, nor, in the case of infants, of the sex to which they should be assigned.

To illustrate the usefulness of this procedure, two patients with the syndrome of gonadal dysgenesis will be presented, an infant 4 months of age and a 15-year-old girl. These two patients demonstrate certain clinical features which facilitate recognition of this syndrome and illustrate the use in diagnosis of the determination of “cytologic sex.” In contrast to their completely feminine appearance, both patients are “chromosomal males.”

Numerous terms have been applied to this syndrome in the past, such as ovarian agenesis, Turner’s syndrome, and the Bonnevie-Ullrich syndrome. As the majority of such patients appear to be chromosomal males and because rudimentary gonads without germ cells are present, “gonadal dysgenesis” is a preferable term for this disorder.

The clinical features are usually striking and clearly defined. In some cases, the association of specific congenital defects makes it possible to suspect the diagnosis early in infancy. These patients are short and have a diversity of congenital anomalies. The configuration of the external genitalia is female but female secondary sexual characteristics fail to develop. The most common anomalies are webbed neck, skeletal and renal malformations, coarctation of the aorta, lymphangiectatic edema of distal portions of the extremities, high-arched palate, microthelia and ocular defects. Mental retardation is an occasional finding.

Patients with this syndrome look very much alike. They usually have flat facial features, some degree of hypoplasia of the mandible, broad chest, stocky build, and short neck with low hair line. Webbed neck, originally described as an integral part of the syndrome, is present only in about one-third of the cases. Bone and dental development are normal or slightly delayed. The short stature is of the primordial type.

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Fig. 1. (a, upper) Photomicrograph of epidermis of a female. The arrows indicate the sex-chromatin mass within the nuclei. As the sex chromatin may be located in different planes within the section, each nucleus must be examined in various depths of focus. (b, lower) The epidermis of a male. A comparable mass of chromatin is rarely seen. (Hematoxylin and eosin.)
None of the secondary sexual characteristics caused by the secretion of estrogen at puberty occurs, but they do develop sexual hair. The female external genitalia, the vagina and uterus are infantile. The sexual infantilism is caused by a primary gonadal defect and not by a deficiency of pituitary gonadotropins. Consequently, increased amounts of gonadotropins are excreted in the urine. We have not observed elevated titers of urinary gonadotropins (in excess of 50 mouse units per day) before 9 to 12 years of age.

The first patient with gonadal dysgenesis is a 4-month-old infant with lymphangiectatic edema of the type described by Ulrich. She was referred to Babies Hospital at 3 days of age because of an unusual degree of edema of the hands and legs, first observed at birth. The family history was unrevealing. She is the first child of young, healthy parents. Pregnancy and labor were uneventful and the vertex delivery was spontaneous. She weighed 3480 gm at birth.

The findings on physical examination now are similar to those found at the time of admission except for a moderate decrease in the edema (Fig. 3). The lower extremities extending up to the mid-thigh are swollen, particularly the dorsum of the feet. This is less on the right than the left. The swelling over the dorsum of the feet pits slightly with pressure. The hands are puffy in appearance, most noticeably over the dorsum of the phalanges. She is of average size for her age (retardation of growth may not be apparent during the first 6 to 12 months of life).

The neck is short and the scalp hair extends to a low level. There are loose longitudinal folds of skin over the nape so that the infant can be picked up like a kitten. In addition, the infant has a high-arched palate, low-set deformed ears, a triangular-shaped mouth, cubitus valgus, and hypoplastic nipples. The blood pressure has been normal in both arms; the femoral pulses are easily palpated.

Urinalysis, concentrations of proteins and nonprotein nitrogen in the serum and the electrophoretic pattern of the plasma proteins were all normal. Because of an increased incidence of renal anomalies, an intravenous pyelogram was done but did not show any abnormalities.

In this infant, the edema of the extremities, the loose skin over the nape, and the multiple anomalies suggested the diagnosis of gonadal dysgenesis. We therefore obtained an oral mucosal smear and a specimen of skin; the nuclei in both tissues showed a male chromatin-pattern. These findings established the diagnosis of gonadal dysgenesis. In the past, unless exploratory laparotomy or peritoneoscopy was performed, it was not possible to prove the diagnosis before the age of puberty, at which time a high titer of urinary gonadotropins could be detected. It is of interest that about 80% of these individuals are "chromosomal males." Our series includes seven infants in whom the diagnosis was made before the age of 2 months. We now believe that the finding of a male chromatin-pattern in a suspected case, irrespective of age, is strong presumptive evidence of the diagnosis; on the other hand, a female chromatin-pattern does not exclude gonadal dysgenesis.

The second patient is a 15-year-old girl who also had edema of the extremities at birth. Because of the swelling, shoes could not be fitted for this child until she was 18 months old.
Fig. 3. (a, above; b, left) Case 1 at 7 days of age.
old. The edema gradually subsided, first the hands and later the feet, and by 3 years of age had almost completely disappeared. Since early childhood she has been conspicuously short. She is now 55 inches in height, and growth has ceased. This is about the average stature which these patients achieve. The habitus is typical (Fig. 4a). The mandible is hypoplastic, the chest broad and the build is stocky. There are bilateral epicanthal folds, cubitus valgus, multiple pigmented nevi and dystrophic appearing nails. The skin over the phalanges of the hands and feet has a characteristic puffiness, the residua of the earlier edema.

At 12 years of age the diagnosis of gonadal dysgenesis was suspected. Urinary gonadotropins were assayed and a high titer detected (greater than 50 mouse units per day). No female secondary sexual characteristics had developed. When the diagnosis was confirmed by the gonadotropin assay, an estrogen preparation (Premarin*) was administered in a dose of 2.5 mg, at first daily and later for the first 21 days of each month. After 6 months of therapy, the breasts were well developed, the vaginal mucosa showed the effects of adequate estrogen, and menstruation regularly occurred during periods of estrogen withdrawal (Fig. 4b). It is advisable to institute estrogen therapy between 12 and 14 years of age, usually in conformity with the patient's wishes, to avoid the psychologic problems of sexual infantilism.

A skin biopsy and a smear from the oral mucosa obtained at 14 years of age showed cells with male-type nuclei.

From the practical standpoint, cytologic examination for sex provides a method for

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Fig. 4. (a, left) Case 2 at age 11 years and (b, right) at 13 years, after 9 months of estrogen therapy.
establishing the diagnosis of gonadal dysgenesis at any age, when a male chromatin-pattern is found. The especial usefulness of this technique in preadolescent cases has been indicated in the two patients presented. Early diagnosis is of more than academic interest. It enables the physician to discuss prognosis with the parents in concrete terms. Once the diagnosis is confirmed the parents can be told that the child will be a short, but not dwarfed, adult. It is important to emphasize that the retarded growth is not amenable to treatment. They should be informed that the sexual infantilism can be treated with female sex hormones at an appropriate age, but that the patient will be sterile. The patients and their families should not be informed of the results of determination of "cytologic sex" in order to avoid any misconceptions of the significance of such findings or adverse psychologic effects which may result from this information.

The results of determination of "chromosomal sex" in gonadal dysgenesis also have theoretical implications, notably a bearing on theories of human sex differentiation. Wilkins and Fleishmann showed that in this condition a true gonad is not present, but in the mesosalpinx parallel to each fallopian tube there is a narrow, white streak, the vestige of the primitive genital ridge. The ridge is composed of connective tissue arranged in swirls, resembling ovarian stroma, covered by a single layer of epithelial cells (Fig. 5). Deeper in the connective tissue varying amounts of mesonephric vestiges, such as rete tubules and small clumps of cells resembling Leydig cells, can be found. Ova, primordial follicles and seminiferous tubules are absent. Thus, the gonads in these individuals are rudimentary and functionless. The male sex-chromosome constitution found in the majority of these phenotypic females provides evidence that there is also an absence of fetal testicular function during embryonic and fetal life.

Fig. 5. Microscopic appearance of the genital ridge of a chromosomal male with gonadal dysgenesis, displaying the absence of germinal elements, the wavy gonadal stroma, and a few mesonephric vestiges. (Hematoxylin and eosin.)
Jost, a French biologist, has performed a series of experiments in rabbits which bear directly on the discordance between phenotypic and chromosomal sex in the "chromosomal males." Jost studied the effects of castration of young rabbit fetuses, beginning at a stage when the testes could be identified but the other genital structures were still indifferent. Following castration, the fetuses were returned to the uterus and allowed to develop to term. These experiments demonstrated that: a) in the absence of gonads, female fetuses always develop normally as females; b) when the fetal testes are removed at an early age, the genital ducts, the urogenital sinus and external genitalia always differentiate along female lines. Jost further showed that the fetal testis exerts its masculinizing effect over a rather short, critical period. Castration of male fetuses at later stages, after duct differentiation had begun, resulted in a progressively less feminine genital tract. At a still later stage, castration no longer modified male differentiation. These and other experiments suggest that there is an inherent tendency of the accessory sex structures of the fetus to develop along female lines. The neutral form of sex differentiation appears to be the female.

The findings in patients with gonadal dysgenesis are entirely compatible with this concept. Patients with this syndrome may be considered the human counterparts of the male and female rabbit fetuses of Jost, castrated at an early stage of sex differentiation. In the human, as well as the rabbit, absence of a functional gonad early in fetal life apparently results in the development of female accessory sex structures, irrespective of chromosomal sex.

Chromosomal males with gonadal dysgenesis illustrate the important role of the fetal testis in human sex differentiation. These patients should be regarded as the most severe form of male pseudohermaphrodisim, that is, genetic males who are anatomically completely feminized in somatic sex development during the fetal period. One can explain the various forms of ambisexual development found in the classic forms of male pseudohermaphrodisim on the basis of varying degrees of testicular insufficiency during fetal development.

CHAIRMAN RILEY: Are there some questions?

DR. ELENA BODER (Los Angeles): Could you say a few words about Turner's syndrome in boys?

DR. GRUMBACH: There is a group of boys, and also of girls, with a number of the congenital anomalies described by Turner and Ullrich (often including webbed neck) in whom testes or ovaries are present. These individuals do not have gonadal dysgenesis. Their chromosomal sex is the same as their somatic sex. They develop, at least in some degree, appropriate secondary sexual characteristics at puberty. The term Turner-Ullrich syndrome might be used for this group, to differentiate it from the syndrome of gonadal dysgenesis.

VOICE: Have studies of sex chromatin been made in rabbit fetuses to learn whether the presence or absence of the gonad makes any difference in the results?

DR. GRUMBACH: In the rabbit, the sex chromatin cannot be distinguished by Barr's technique from other chromatin masses within the nucleus. However, the sex chromatin mass has been identified in early human and cat embryos prior to differentiation of the gonads and in syncytiotrophoblastic cells of a human blastocyst. Recently, the cytologic sex of human fetuses has been determined by the chromatin-pattern of cells contained in amniotic fluid.

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