DISCUSSION
Fig 47A: Karyotype with 46,XY chromosomes from patient no 163/78

Fig 47B: Photomicrograph of cultured leucocytes of case no 163/78 in mitotic metaphase. This cell is the same as the one analysed in Fig 47A.

Fig 48A: Karyotype with 47 XYY chromosomes with chromosomes of patient no 10/78

Fig 48B: Photomicrograph of cultured leucocyte of case no 10/79 in mitotic metaphase. This cell is the same as analysed in Fig 48A.

Fig 48C: Karyotype with 46 XY chromosomes from patient no 10/78
Fig 49A - Karyotype with 46 XY chromosomes from patient no. 194/78.

Fig 49B - Photomicrograph of cultured leucocyte of case no. 194/78 in mitotic metaphase. This cell is the same as the one analysed in Fig 49A. Carbol fuchsin stained.

Fig 50A - Karyotype with 46 XY chromosomes from patient no. 181/78.

Fig 50B - Photomicrograph of cultured leucocyte of case no. 181/78 in mitotic metaphase. This cell is the same as the one analysed in Fig 50A. Carbol fuchsin stained.
Incidence

The incidence of sex chromosomal aberrations has been studied in the newborn, in the normal adult populations as well as in cases suffering from specific diseases (see Review I page 26, to 49 to 53 and Review II pages 49 to 53). In the newborn populations, it is well known that alteration in the maternal hormone levels may produce changes in the size and visibility of the sex chromatin, resulting in a high percentage of negative cells (Schmidt et al. 1965, Brainerd et al. 1966). This might account for the low positive findings of certain workers observed in the past in this country (Naik and Shah 1962). It is customary, therefore, to repeat buccal smears at a later date of life for confirmation. Hence sex anomalies should not be diagnosed without proper karyotyping.

In India screening of newborns has yielded aberrations in about 0.68-0.8/1000 female births comparable with 0.6/1000 births reported by Hamerton (1972) for the world.

Usually sex chromosomal aberrations are found in much higher numbers in select cases suffering from specific syndromes related to skeletal or sexual developmental errors. Thus hypogonadism, primary amenorrhoea, ambiguous external genitalia, short stature and mental retardation in this series show a high degree of sex chromosome aberrations (see Table 1.2 of this thesis).
In the present study the females have been classified (see Table III.1) into the typical Turner phenotype with complaints of short stature, webbing of the neck and other stigmata including primary amenorrhoea. There were a total of 11 cases of which five (45.4%) were X positive and six X negative (54.5%). Primary amenorrhoea with minimal hypogonadism or infantilism accounted for the largest number of cases. 79% of them were X positive, 12.7% were X negative and 8.3% showed low X positivity. Secondary amenorrhoea, usually associated with one or two so called normal periods without Turner's symptoms was recorded in 19 cases of which 78.9% were X positive, 10.6% X negative and 10.6% low positive. Out of ten cases of hirsutism two showed X low positive nuclei. Three cases were diagnosed as testicular feminisation. Of them two were X negative and one X positive. The second largest group of cases (68) was associated with short stature, mental retardation and infertility. Of these again, 76.5% were X positive, 19.1% X negative and 4.4% X low positive. Apparently, therefore out of 276 phenotypic females, sex chromatin abnormalities were observed in 70 cases (25.4%). This percentage of abnormalities is rather high and may possibly be attributed to the selection of the cases.

In the phenotypic males (see Table III.2), 55 had normal sex chromatin and 15 an X positive pattern. The X positive ones included three Klinefelters syndrome (4.2%), three gynaeo-
mastia (37.5%), two hypospadias and six cases of short stature, mental retardation and infertility. Patients with hypogonadism and cryptorchidism, on the other hand showed much lower frequency of X positive cases. There was no incidence of X positive chromatin in the cases of cryptorchidism studied and of 21 cases of hypogonadism, only one (0.76%) was X positive.

Thus, a study of the sex chromatin pattern is perhaps one of the best methods of screening known for chromosomal aberrations, provided a strict control is maintained. It serves to identify the genetic complement among the large number of cases attending an average referral unit for various endocrine and gynaecologic complaints involving aberrations of skeletal, mental and sexual development.

Alterations in hormone levels were also observed to influence the size and number of the chromatin bodies in the present investigation, supporting earlier evidences (Blanco et al. 1965, Schmidt et al. 1966, Chakravarty et al. 1978). Thus cases of short stature or hypogonadism due to other hormone deficiencies, such as thyroidism or adrenalism, may also display low sex chromatin levels, making it necessary to exclude external hormone therapy in these cases.

Relationship with age:

In the majority of cases studied (70.2%) the age of diagnosis in the males and females was 11-20 years (see Table III).
The next most common group amongst the females was 21-30 years (66 cases) while in the male (0-10 years) there were 16 cases. In the female (0-10 years) there were 12 cases. In the age group 31 years and above the males presented 16 cases while the females gave four. These observations indicate that the majority of individuals became conscious of their disease only at puberty. 176 of 276 females came at 13 years or later with various complaints, of which the main appeared to be the absence of secondary sex characters and amenorrhoea. 2 cases were about 10 years of age and complained of short stature. On the other hand, sterility appeared to be the common factor in the older age groups.

In the case of the males, the date of reporting was even earlier, hypogonadism being the most frequent symptom at an early age. While it is well known that Turner's syndrome may be diagnosed in the infant due to abnormal facies, loose skin and lymphoedema such cases are rarely diagnosed early in our country.

The cases that came at an early age were those of hypospadias, cryptorchidism and ambiguous external genitalia, probably due to the fact that such symptoms are more obvious earlier. A better consciousness of the phenotypic changes occurring due to chromosomal aberrations might bring them earlier. Some cases are obviously diagnosed only at puberty, e.g., testicular feminisation, but there is no justification of diagnosing the Kline-
fetters after 31 years of age. The value of good perinatal and infant care in our country therefore cannot be overemphasized (see Review I for references).

**Sibship:** Table III.13 shows that of the female patients studied here, two were the only children and the rest were distributed variably among the sibs. In the males three individuals were the only issue while the remaining had one or more sibs. It has been observed that, in cases of major chromosomal aberrations as well as congenital defects, parents do not go in for another pregnancy. However, the relative lack of symptoms detectable early in life does not have the same effect in the case of sex chromosomal aberrations. Amongst cases of proved chromosomal aberrations only two males were the sole offspring while there was no relation to sibship for all the other cases. 15 females had four more sibs while only one male had four or more sibs. Only five females were the last in the sibship. Thus sibship does not appear to have much effect on chromosomal aberrations.

**Relationship with caste or social groups:**
This being a referral hospital, cases were seen from all over the city as well as neighbouring States. Data on these aspects in our country is still inconclusive. There was no clustering of any particular caste or religion in the cases of sex anomalies studied here (see Table III.14). The higher number of Muslims (14.7%) may or may not be significant related to
their percentage in the general population.

**Enzyme studies:**

Studies on the activity of the marker gene coding for glucose-6-phosphate dehydrogenase and the nature of the chromosomal anomaly detected failed to show any significant correlation between the gain or loss of X chromosomes and the enzyme level (see Table III.17). For earlier work carried out in India on these lines, please see pages 53 to 56 of this thesis.

**Associated factors:**

(a) Physical development: It is usual in India to consider $151.7 \pm 10.24$ at 18 years to be the parameter for lack of skeletal growth of a female. In the individuals studied here this standard has been followed (ICMR Report, 1972).

In general, cases of Turner's syndrome as well as some mixoploids belonged to this category while individuals with testicular feminisation, pure gonadal dysgenesis and primary amenorrhoea were above this height. The Indian figures for height, especially for rural and low urban classes, tend to be low (ICMR Tech. Rep. Ser. 18, 1972; Trivedi, 1977). The causes are obviously dietary deficiency or systemic diseases. In the present study hypothyroidism may also have played a part as associated hypofunction of the thyroid has been reported from this part of the country in other chromosomal aberrations (Sadhukhan 1978). The presence of increased thyroid auto-
anti-bodies in sex chromosomal disorders has also been well documented (Hamerton 1971). These were found to be highest in mosaic Turner's as compared to the normal. The lower part of the body (below the symphysis) was found to be longer than the upper half in all cases and the span equal to or more than the height (see Table III.15).

(b) Mental development: Although 68 cases among the females and 28 of those with the male phenotype complained of mental retardation, chromosomal information was available only in a few patients. In cases with definite karyotypic defects, mental retardation was found, as expected, to be associated with Klinefelter's syndrome and triple X. However, some patients, complaining of minimal hypogonadism with primary amenorrhoea, showed mental retardation of a minimal type. The cause in such cases is apparently unrelated to the genetic complement. Individuals with testicular feminisation did not exhibit any such symptoms.

(c) Sexual development: As mentioned earlier in this discussion, lack of secondary sex characters was most marked in hypogonadism and Turner syndromes. The cases of primary amenorrhoea with minimal hypogonadism in 79% of the X positive cases showed mostly normal height and normal sexual development. However, development of hair in the pubic and axillary region was often absent. The 68 patients with short stature and sexual infantilism showed in 18.8% cases signs of mulle-
rian agenesis. Obviously menarche was delayed or absent in such cases. Although some were recorded as familial, in the majority there was no family history.

19 cases of secondary amenorrhoea exhibited normal secondary sex characters. Of these, only two were X negative (10.6%) and two (10.6%) X low positive. Six cases of primary infertility with no hypogonadism were included in the present survey of which one (16.6%) showed X negative and four low positive nuclei. Of the males, majority (29.5%) complained of hypogonadism. Eight cases, in addition, had gynaecomastia, unilateral or bilateral. It is interesting that, of these, three were proved to be Klinefelters. Hirsutism was reported in 10 females of whom two showed low X positive while 80% had X positive nuclei. Similarly the adrenogenital syndrome was reported in a phenotypic male with hirsutism at eight years of age but found to be a karyotypic male.

Results of karyotypic studies:

The karyotypic data (Table III.3 and 4) in the case of 33 selected phenotypic females showed eight cases of 45 XO and five of 45X0/XY mosaicism. Most of them exhibited the typical phenotypic changes like short stature, webbing of the neck, short third metacarpal, absence of secondary sex characters. The uterus was found to be rudimentary and streak gonads were observed in those cases where laparoscopy was carried out. In most cases of Turner's syndrome the tri-iodothyronine (T3)
and thyroxine ($T_4$) levels were also low. The follicle stimulating hormone (FSH) level was less than one in most cases whereas Turner mosaics showed higher FSH levels. A comparison of the XO and the mosaic group however showed no significant differences in the clinical features of the patients.

**General:**

It is difficult to obtain a satisfactory figure for the incidence of ovarian dysgenesis in the general population. In the present series $X$ negativity was found in 9.4% of the total number of cases. Chromosomal abnormalities, on the other hand, were found in 45.4% of the individuals tested specifically for suspected karyotypic abnormalities (33 cases). Thus a large percentage of cases showed signs of primary gonadal dysgenesis without karyotypic abnormalities, 17 cases, in fact, exhibited the normal 46,XX pattern in spite of very marked phenotypic changes.

The patients with primary amenorrhoea therefore may be divided into two main groups, those with an abnormal chromosome constitution or sex chromosomes in disagreement with the phenotype (e.g., testicular feminisation) and those with the normal 46,XX constitution.

Pure gonadal dysgenesis (Swyer's syndrome), comprising of primary amenorrhoea with streak gonads and hypogonadism, has
been reported to be familial (Espiner et al. 1970) due to a sex limited or autosomal gene (Sarto 1974). Absence of any male inducer hormone and the difference in phenotype are suggested to be due to different degrees of penetrance of the mutant gene (Hamerton 1971).

In the series here there were only two such cases who gave a history of other female sibs being similarly affected. On the whole, cases of pure gonadal dysgenesis do not suffer from skeletal defects as much as those with hypogonadism and eunuchoidism. Our patients were on an average relatively tall but had gonadal streaks and the internal genitalia were infantile. All the cases of pure gonadal dysgenesis studied here were observed to have the XX constitution.

The normal XX constitution was found in primary amenorrhoea cases with vaginal defects and usually no chromosome abnormalities have been found in such cases previously (Azoury and Jones 1966; Sarto 1974). Developmental abnormalities of the vagina and/or uterus is a common cause of amenorrhoea. In the cases with rudimentary uterus and/or absence of vagina the XX constitution was found. Another case with imperforate hymen and mullerian agenesis also had the XX constitution.

The cause of XX gonadal dysgenesis may be genetic or environmental leading to a blockage at the foetal stage (Hamerton 1971). An undetected y - line has been suggested (Hamerton, 1971). A mutation at an X-locus or translocation of
y have also been said to be the cause (Polani 1972, Boczkowski 1973). Gonadal dysgenesis with eunuchoid characters and lack of secondary sex characters, feminine external genitalia, internal genitals showing rudimentary tubular structures have been reported. Nevertheless this syndrome has rarely been recorded in families (see Review II of this thesis).

The testicular feminising syndrome, reported to be present in 1/2000 to 1/20,000 of the population (Sergovich 1976), has received considerable attention due to its unexplained phenotypic changes (see Review II of this thesis). Two cases in this series were diagnosed as having testicular feminisation. None, however, displayed a familial inheritance pattern, such as a sex linked trait. It is believed that in such individuals testosterone is not converted into dihydrotestosterone due to target cell insensitivity. This may perhaps be due to a change in the autosomal locus responsible for the formation of testosterone from the primary steroids on the basis of the fact that autosomal determination of the testicular feminisation syndrome is known in man and animals (McFeely 1967, Hamerton 1969, Lyon and Hawkes 1970). Thus the cause of primary amenorrhea in such cases is not due to the chromosome constitution as such but to gene mutations which may influence sexual development.

Karyotypes of phenotypic males studied here showed 57.1% abnormalities (Table III.5,7). This may be ascribed to the
ease in selection in most of these cases. The percentage of Klinefelter's syndrome was highest. One case was pure and four were mosaics. Two cases showed the 46XY karyotype. The frequency of Klinefelters and XYY determined from buccal smears was also high and a large number of these cases are often not required to be karyotyped. The majority of these cases complained of hypogonadism and other stigmata like gynaecomastia and sterility. The two cases karyotyped (Table III.5) and found normal showed ambiguous external genitalia. One of them, although reared as a male, yet had very atypical external characters and impalpable testis. This child was three years old and demonstrated infantile levels of FSH and LH ($< 1.0$, $< 1.0$) and 6.7 prolactin. $T_3$ was 0.9 and $T_4$ 48.9 ng /ml.

The value of proper assignment of sex in these cases and reconstitution surgery is obvious.

Karyotypic investigation of cases with ambiguous external genitalia, hirsutism and pseudohermaphroditism rarely displayed mosaicism, the karyotype being either XX or XY. This observation suggests that the development of the external genitalia and subsequent feminisation or masculinization are indirectly governed by the sex chromosomes. The X or Y chromosome, concerned with sex induction, would normally initiate the formation of the primary germ cells and their migration to the genital ridge and perhaps their maturation to primary oogonial or spermatogonial cells. The surrounding mesodermal cells that give rise to the hormone stimulating follicle cells
are probably governed by other genes which may be located on the autosomes. These may, in turn, interact with the genes regulating the function of the adrenal and hypothalamic or pituitary axis, leading to the consequent development of the male or female phenotype (Boczowski 1971, Bühler and Stalder 1977). This may perhaps be an explanation of the considerable overlapping of the phenotypic and genotypic characters. Careful karyotyping is thus required for distinguishing between the cases of primary genetic (Turner's syndrome) and primary gonadal hypofunction.

According to Polani (1972) cases of gonadal dysgenesis represent a failure at some stage of secondary sex development. Does this suggest that the second line of gonadal development involves the X chromosome rather than the autosomes? The latter may carry contributory genes necessary for steroidal metabolism and the hypothalamic pituitary axis. The cases of reverse XX pure gonadal dysgenesis could represent cases where the X chromosome genes activated by the Y product are completely absent while testicular feminisation presents an incomplete or less penetrant form of the syndrome. Other theories regarding the cause of testicular feminisation are (i) deficient Y allowing initiation but not development of primary germ cells, (ii) an allele for producing female hormones present in the X and probably not suppressed by the male producing gene and (iii) autosomal control of hormones at any step at
which an allele may be altered causing a change in the hormone synthesis.

It is well known that steroidal hormones are responsible for skeletal growth and development (see Review II of this thesis). The influence of the X chromosome complement on skeletal growth has already been noted and the excessive height of Klinefelters attributed to it (Hamerton 1971). This has not been related directly to the number of extra X chromosomes for the triple X female is skeletally retarded. Moreover, a single pair of genes is not likely to be responsible for skeletal development as also the relative mass of heterochromatin per se. A series of interactive genes on the X, Y and autosomes may be hypothesized to regulate stepwise organisation of the complete sexual development in the male or female and the possibility of polygenic control cannot be ruled out. This could well explain the relative confusion of varying types of hypogonadism with only a few well documented cases of dominant or recessive transmission. A block at any point would produce retardation, sexual, mental or skeletal. That the problem in complex is clear, especially as the psychic brain function is also considered for whereas testicular feminisation or the Turner's syndrome is usually of normal interest, the triple X is not and the Klinefelter is more liable to be less aggressive compared to the XYY male (Akesson and Walhstrom 1977).
An overall assessment of the sex chromosomal studies shows no precise relationship with external environmental factors, like diseases, exposure to radiation or drugs. Though such reports are available yet they are few and far between (Robinson and Puck 1969, Stoller and Collman 1965, Hecht et al. 1964, Heinrichs et al. 1963) and deal principally with autosomal anomalies. Since the cases studied here came at a much later age, it was not possible to trace the karyotypic abnormalities to prenatal influences. A survey of newborns, from this aspect, would be a promising field of work.