CHAPTER-III : REVIEW OF LITERATURE

HISTORY OF DEVELOPMENT OF GENETICS

The hindu scriptures show that the ancient Indians had the knowledge about the conception and the birth of a child. Atharva Veda Book III (edited by Federick Maxmueller, 1962) has mentioned that male and female parents are needed for the birth of a child.

In the Sankhayan Grihya Sutra (edited by Federick Maxmueller, 1964) mentioned that son borns after 10 months. It has also mentioned that the sperm of a man pours into a woman.

Determination of the sex of the offspring was also mentioned in the Manu Samhita (translated by George Buhler, 1970). At this time genetic role of the mother was clear. In those days people were also knowing about the heredity influences on phenotypic as it has advised to look to pedigrees.

The Manu Smriti (translated by George Buhler, 1970) advised the people not to marry with person whose family members gave the history of epilepsy, extra body parts, physical defects etc. and also prevention by avoidance of cousin marriages.

The ancient Babylonians pollinated date palms with pollens from a sturdy parm tree with the desired quality.

The Jewish Talmud points towards the practical use of genetical knowledge in man. It lays down that circumcision should be avoided in children whose elder brothers and maternal uncles have bleeding disease (cited by Niyogi and Srivastava, 1986).

REVIEW OF LITERATURE

From the ancient Hindu Pandits to the illustrations of Greek Philosophers all in their famous treatise, text or Vedas have mulled and amused over the possibilities of inheritance of certain characters from generation

to generation. Some of them even considered the possibility of inheritance of few diseases. What they could not think of was method or medium of transfer of these characters in the offsprings. Basically, they were also ignorant about the microstructure of man or any other living animal.

Ideas about heredity can be traced back at least 6000 years by means of stone engravings from Chaldea which depict pedigree concerning the inheritance of certain characteristics of the mane in horses. With regard to human heredity, the inheritance of human disorder haemophilia was mentioned in Talmud some 1500 years ago (cited by Emery, 1988).

Aristotle (300 B.C.) suggested that male semen originated from the blood and possessed the ability to give life to the embryo which was formed in the uterus by coagulation of menstrual blood. This idea was generally accepted for nearly 2000 years until when William Harvey (1700) who had studied the blood circulation demonstrated that in deer killed at various times after mating there was never any evidence of coagulation of menstrual blood but that a small embryo developed which gradually increased in size and complexity throughout the whole period of gestation, a possibility foreseen in the Koran, the Holy Book of Muslims, several centuries years earlier (cited by Emery, 1988).

Regnier de Graaf (1641-73), Dutch Scientist, was the first to recognise that the union of female egg and male sperm is the essential nature of conception. For the first time, the idea was put forward that the sperm alone was not the sole heredity agent out of both male and female parent transmitted characteristics to their offsprings (cited by Emery, 1988).

Maupertuis (1689-1759) born in France stated, certain hereditary traits in man such as extra fingers (polydactyly) and lack of pigmentation of the hair and skin (albinism) and from pedigree studies showed that these two conditions were inherited in different ways. His conception of the structural basis of heredity particles; each particle was destined to form a particular body part and each body part was formed by the union of two such particles; one from one parent and one from the other. One

particle might dominate the other and so the offspring would come to resemble one parent more than the other. Robert Hook (1665) was the first to see the cells of cork (cited by Emery, 1988).

More than a century ago, Gregor Mendel - "Father of the Modern Genetics" (1822-1884), an Austrian monk, by his painstaking experiments on pea plants made careful observations on the mode of transmission of physical characters from one generation to other, which have stood the test of time. These observations went unnoticed even by Galton and Darwin until they were rediscovered in early 20th century by three investigators independently of each other. Garrod (1902), in his classical Croonian lectures on the "Inborn Errors of Metabolism" proposed that genes act through their effects on enzyme synthesis.

Human chromosomes were first seen in dividing cells (Virchow, 1857), although their true nature was not recognised then. In 1874, the Hungerian Scientist, Von Torok, observed the stages of mitosis. The detailed studies of human chromosomes, however, were carried out in 1879, by the German anatomist, Arnold. In 1882, the term "chromatin" was coined for the stainable portions of the nucleus by Flemming. Shortly after that, Weismann (1883), Strasburger (1884) and Von Kollicker (1885) independently concluded that the chromatin was the physical basis of inheritance. The term "chromosome" was introduced for the same entity by Waldeyer in 1888.

Between 1910 and 1930, the principles governing the maintenance and equilibrium of genes in populations were enumerated by Hardy, Weinberg and others. At the same time, important advances were taking place in microbial genetics. Beadle and Tatum proposed "one gene - one enzyme" theory.

FAMILY HISTORY AND MENDELIAN PATTERNS OF INHERITANCE

The importance of family history in medical practice has long been recognised. Normally one inquires only regarding family history of infectious diseases, hypertension, diabetes, etc. With a better

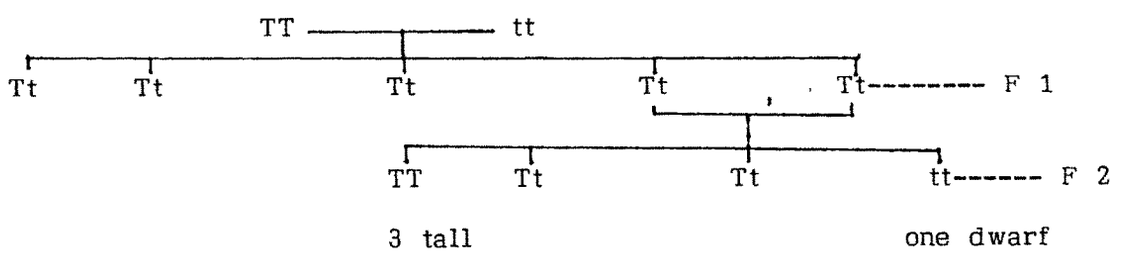
understanding of the genetic contribution in several common conditions such as schizophrenia, congenital heart disease, cleft lip and palate, mental retardation, etc. a proper family history assumes a greater importance. The record of the family history in the form of the pedigree can be an efficient form of the record keeping. A good family history can even save a patient's life. For example, in case of malignant hyperthermia, if one of the parents or sibs has had the condition, the family physician can alert anaesthetist not to use certain anaesthetic agents. At this stage, it is important to introduce the classical Mendelian principles of inheritance. An understanding of these can help one to counsel his patients and their families with a greater degree of confidence for the conditions inherited in a Mendelian way.

From his painstaking experiments on garden peas, Mendel anunciated the principles of heredity in the form of two laws :

1. First Law or Law of Segregation of Alleles

It states that characters are controlled by pairs of heredity factors which segregate from one another during the formation of germ cells. In an individual, each number of an allele pair is originally derived from separate parents, one from male and other from female. The traits or characters determined by such pairs of alleles do not blend in the offspring, since the alleles retain their identity and can pass unchanged from one generation to the next, irrespective of the expression of a trait in any generation.

e.g. Tall characters could be represented by T T in one parent - Male
e.g.Dwarf characters could be represented by tt in other parent - Female



If these factors (TT) are the same, then the individual is said to be homozygous, but if these factors are different then the individual is said to be heterozygous (Tt). In the heterozygous state a character which is manifest is "dominant" one which is not manifest is "recessive". Johannsen gave the word gene for these heredity factors. The genes responsible for contrasting characters are referred to allelomorphs or alleles for short. Thus, there are two alleles for stature, one for tallness and one for shortness.

2. Mendel's Second Law of Inheritance or Law of Independent Assortment

This law states that during gametogenesis members of different gene pairs assort randomly or independently of one other. While Mendel's first law is concerned with the behaviour of one pair of genes during gamete formation and fertilization, his second law defines the relationship between different pairs of genes in the same individual. It deals with dihybride cross, where two pairs of genes are involved. If one of the parents is homozygous for two genes with dominant traits (AABB) and the other is homozygous for the recessive alleles of the same two genes (aabb), then all the F1 offspring will be heterozygous for both of the genes (AaBb). During meiosis, four possible kind of gametes of F1 individuals are produced i.e. AB, Ab, aB, ab. If we crossed two F1 individuals the possible combinations would be a dihybrid cross with ratio 9:1:3:1 (1 has both dominant and 1-ab, recessive). It can be seen that each of the genes segregate from each other and pair in an independent fashion giving rise to new combinations of characteristics. This is Mendel's second law.

Similarly, a person with polydactyly (a dominant factor in man) has an equal chance of transmitting to any particular child either the gene for polydactyly or the gene for normal hands. Each child does not receive from one parent a little bit of each. It is extraordinary that Mendel formulated these ideas without any knowledge of these hereditary factors or gene. Walter S. Sutten (1903) and Theodor Beveri (1903) independently proposed the chromosome (Chroma - colour, Soma - Body) theory of

heredity, the association between these minute structures and the phenomenon of inheritance. Emil Hoitz (1936) showed some longitudinal differentiation of chromosomes in to euchromatin (genetically active) and heterochromatin (genetically inert).

It was found that the chromosomes occur in pairs in the somatic cells. The members of one pair called homozygous chromosomes; one is of maternal and the other of paternal origin.

The importance of the chromosomes as the site of hereditary traits was not recognised until the end of the 19th century. It was then learned that the number of chromosomes in each cell and the shape of the individual chromosomes are constant and characteristic for a given species.

Apparently, Hansemann (1891) was the first investigator to attempt the determination of the number of chromosomes in human cells. Wienman (1917) reported the presence of X and Y chromosomes in the human and one year later Evans (1918) described 48 chromosomes in the human somatic cells.

Painter (1921) saw 46 chromosomes in the clearest metaphase plate, but two years later, he reported that 48 was the characteristic diploid number in both human sexes. This was the general belief until when Tjio and Levan (1956) reported that they found 46 chromosomes in human somatic cells. The observation was quickly confirmed by Ford and Hamerton (1956) in preparations of semi-niferous tubules. Other investigators proved this in bone marrow cells (Ford et al, 1958) in leukocytes from peripheral blood (Hungerford et al, 1959) and in the cells from skin and fascia (Tjio and Puck, 1958). It is well established now that the basic chromosome number in man is 46; the female has an XX and the male an XY sex chromosome complement. Although some observers thought that testicular tissue may show an abnormal chromosome number or fragmentation (Kodani, 1958 a and b; Stern, 1959), the observations of Makino and Sasaki (1960) did not support this.

Hungerford et al (1959) made a discovery which revolutionized chromosomal analysis in man. They found a substance called phytohaemagglutinin, an extract of the red or pentabean (Phaseillus Vulgaris) when added to the blood and incubated in a nutrient medium, stimulated the peripheral blood lymphocyte to divide. This provided a large number of dividing cells and helped in discovering many abnormalities of the autosomes and sex chromosomes. Over a period of time, minor improvements in the techniques were recommended and this improved the quality of preparations.

Till 1970, the limiting factor in the development of human cytogenetics was the inability to identify more than a few chromosomes in the human complement. However, Casperson (1970) and his colleagues changed the fundamentals of cytogenetics by showing that they could individually identify all the chromosomes by staining them with quinacrine solution. With this, began the era of individual chromosome identification. Soon, a number of new banding techniques were developed. The G banding by Seabright in 1970, C banding by Pardue and Geme in 1970, R and T banding by Dutrillaux in 1973 and N banding by Matsui and Sasaki in 1975, Yunis later on in 1979 added prophase banding.

With introduction of newer techniques the horizon of cytogenetics widened but the lack of unanimity and uniformity created great confusion about reporting of the findings. This led to a series of international conferences, starting from 1960 in Denver to 1971 in Paris for deliberations and determination of a definite, standard terminology.

In 1960, at Denver International Conference, human karyotype was classified into A to G groups and also the methods to identify each group were standardized.

In 1963, the Second International Conference was held at London, where new methods of identification of individual chromosomes were suggested.

In 1966, Third International Conference held at Chicago proposed short hand nomenclature for the karyotype and certain anomalies. With the introduction in banding in 1970, the fourth International Conference was held at Paris, where the human karyotypes were classified in relation to bandings. In 1975, additional recommendations were passed where the sub-bands were given proper number.

Staining for nuclear chromatin bodies is widely used for cellular sex determination with the complement of 2 independent techniques one for sex chromatin and the other for chromosomes, the subspeciality of medical cytogenetics was born.

For the normal ovarian differentiation, both X-chromosomes are necessary. Both X-chromosomes in oocytes are active and code for the X 'linked' genes G6PD and hypoxanthine guanine phosphoribosyltransferase (Epstein, 1972; Gartler and Andina, 1976). In all other cells either maternally or paternally derived X chromosome becomes inactive. Once this inactivation is confirmed same X-chromosome is transmitted to all descendents of that cell. This control system serves as a mechanism of dosage compensation by which female somatic cell functions virtually as if it had only one genetically active X-chromosome (Lyon, 1983). The female therefore, in effect has no more active genetic material than does the male. This hypothesis is known as "inactive X theory", the "Lyon hypothesis". Modification of DNA by methylation is a possible mechanism of segmental inactivation of the second X-chromosome (Reggs, 1975; Mohandas et al, 1983).

Individuals with 45, X and 47, XXX constitutes, for example, have abnormalities both in their sexual development and in somatic features unrelated to sex (Therman et al, 1980). There are two obvious possibilities.

1. The abnormalities may be initiated at an early embryonic stage before inactivation takes place.

2. The original X inactivation may not be regular or random in all cells with abnormal X-chromosome constitutions. Cells with abnormal X chromosome numbers, being genetically unbalanced; would give rise to abnormal phenotypes.

The female germ cells require two active X-chromosomes to give rise to normal oocytes. The X-chromosome must be inactivated prior to meiosis in male germ cells for normal spermatogenesis to occur.

It is estimated that about 80% of the clinically diagnosed patients with Klinefelter's syndrome are chromatin-positive and about 80% of the patients with Turner's syndrome are chromatin-negative (Barr, 1959).

B. AUTOSOMES

Mongolism (G-21-Trisomy Syndrome - Down's Syndrome)

Mongolism is possibly the most frequent and the known congenital anomalies. The earliest description was that of Segun (1846) who coined the term "Furfuraceous idiocy". The term "Mongolism" type of idiocy was given to the condition 20 years later by Langdon Down (1866). Since then, the most commonly used term for this disease has been "Mongolism" and the patient is generally referred to as a "mongol" or a "mongoloid".

The term "Down's syndrome" or "Down's disease" has been used in the Soviet as well as the English literature (Penrose, 1961; Allen et al, 1961).

Among the etiological factors maternal age was the first factor recognised to be important in the etiology of mongolism. Mitchell (1876) and Shuttleworth (1895, 1909) observed that children with mongolism tend to born at the end of sibships. Later view substantiated an increased frequency of births of mongoloid children with increasing maternal age but not with increasing paternal age (Jenkens, 1933; Penrose, 1933; Collman and Stroller, 1962). Penrose, however, suggested that the incidence of birth of children with mongolism is bimodel with respect of

maternal age; one peak is found at a maternal age of 27 and another occurs over the age of 40. Thus, not all cases of mongolism are maternal age-dependent.

Different observations have led to many speculations as to the etiology of mongolism. Before 1959, at least 39 theories were offered to explain the etiology of this disease (Warkany, 1960). Some of the investigators namely Waardenburg (1937), Bleyer (1934), Fanconi (1939) and Penrose (1939) suggested that a chromosomal aberration may be the underlying defect and recommended cytogenetic studies. In 1959, Lejeune et al (1959) reported that the children with mongolism had 47 chromosomes, the extra chromosomes belonging to the group of small acrocentrics. The same was confirmed by others (Ford et al, 1959; Book et al, 1959; Jacobs et al, 1959; Levan and Hsu, 1960), whose reports appeared in rapid succession. The identity of the chromosome found in triplicate was not certain, although it was believed that time to be G 21. While later autoradiographic observations indicate that the chromosome in question is in fact G 22 (Yunis et al, 1965), it is generally accepted that the chromosome involved in mongolism should be called G 21.

The definition of the G 21 chromosome is that which produces mongolism when it is present in a trisomic state (Lancet, 1961).

Assuming normal parental chromosome constitution, G 21 trisomic child can be produced by either meiotic non-disjunction or mitotic non-disjunction during the first cleavage division of zygote (The question then arises to which of the two types of cell division is more susceptible to an aging effect, out of these two types of cell division). The general concensus of opinion is that, mitotic non-disjunction is the principal factor in mongolism.

It should be pointed out that while most of the explanation offered concentrate on meiotic division but aging may interfere with the mitotic division of the zygote as well.

The incidence of mongolism at or shortly after birth was studied in white populations and the values reported range from 0.45 to 3.4 / 1000 births. There seems to be no difference in frequency between mongolian populations and white populations (Lejeune, 1964). Among inmates for mental deficiency, the incidence of mongolism was found to 90/1000 in both Sweden and New York State (Allen and Kallman, 1957).

Mongolism is a congenital disease characterised by a combination of mental retardation and several morphological characteristics. It is essentially a syndrome characterized by number of physical abnormalities of ectodermal origin. A mongol child is supposed to go through a fairly normal organogenetic period in the first weeks of fetal development, but thereafter the development deccelerates and the anomalies of the brain, heart, bones, dentition become apparent. None of the physical features seem to have diagnostic value if taken alone but the association of at least 4 of the most frequent observed anomalies may be regarded as a minimum diagnostic standard (Penrose, 1949). A frequently accepted group of diagnostic characteristics is that given by Oster (1953) which consists of the following :

01. 4 finger line (Simian crease)
02. Short-crooked 5th finger
03. Short broad hands
04. Hyperflexibility of joints
05. Oblique palpebral fissures
06. Epicanthus
07. Furrowed tongue
08. Irregular abnormal sets of teeth
09. Narrow high arch palate
10. Flat occiput.

Gustarson's (1964) criteria for the clinical diagnosis of mongolism include mental retardation plus four or more of Oster's ten "cardinal signs".

There is a great variation in the degree of mental retardation in this condition. Tredgold (1937) found that the majority of his mongoloid

patients were in the I.Q. range of 25 to 45, although an I.Q. upto 74 and a mental age upto 10.8 years have been encountered. Oster (1953) reported that about 4% of the patients in his series could read with comprehension and about 2% of them could write. Mongols have a faculty of minority, sense of rhythm and cheerfulness but at the same time, they may be stubborn. They enjoy eating, singing and listening to music and are good-natured and sociable.

The motor development of these patients is retarded. Most of the children set up by the age of 12 months and learn to walk between 2-3 years of age (Oster, 1953). A few learn connected speech that is, sentence formation by 3 years of age, but most could not from sentences till they were 6 years old. Some never learnt how to form sentences (Schaffer J.A. et al, 1977).

In the brain minor, fissural and gyral deviation have been described and histologically there are minor changes in the ganglion cells as well as areas of defective myelin formation (Schaffer J.A., 1977).

Other physical features that are frequently encountered include flat and broad face, protruding tongue which is sometimes fissured, everted and furrowed lip, hypoplastic maxilla, flattened orbit, peripheral speckling and decrease in stroma of iris, lens opacities, convergent strabismus, nystagmus, prominent ears with folding of upper part of the helix and attached lobe, short and broad neck, susceptibility to infections, thorax deformities, high frequency of congenital heart defects, hypoplastic penis and small or undescended testis in males, over-sized and round labia majora and underdeveloped or absent labia minora in females. Short broad flat and hypotonic hands with short metacarpals and phalanges, absent distal flexion crease on the 5th finger, short and clumsy feet with a well marked gap between the first and second toes; generalized muscular hypotonia, sparse pubic and axillary hair, and sparse growth of beared in males (Gustarson, 1964).

With advancing age, the epicanthus gradually disappears, muscular hypotonia improves and the hyperflexibility of the joints decreases,

while the skin increases in dryness and the hair of the head becomes more sparse and rough.

Mongols are usually born before term (Parker, 1950; Smith and McKenon, 1955) have a lesser birth weight and a lower weight during first year of life. They are shorter than normal when they grow up and very few affected men and women become taller than 155 cms and 145 cms respectively (Oster, 1953).

Changes in the internal organs include brain anomalies, hypoplastic thyroid, hypoplastic adrenals, abnormal thymus, fetal arrangement of glomeruli, megacolon and microcolon and abnormal ovarian function.

Dermatoglyphic Findings

Dermatoglyphic patterns in mongolian were studied extensively by Cummins (1939), Turpin and Casper-Fonmarty (1945), Fang (1950), Turpin and Lejeune (1953), Penrose (1954), Walker (1957, 1958), Rowe and Uchida (1961), Uchida and Soltan (1963) show ulnar loops on most of the finger and radial loops on fingers 4 and 5. In the palm , there is distal axial triradius or large 'atd' angles. The soles show arch, triradial or small loop distal in hallucal area. A marked crease between first and second toes may be present. A simian crease is usually present and also crease on finger 3.

Regular trisomies comprise of 95% of the patients with mongolism. Approximately 1% are mosaic (this is doubtless a minimum since some of the mosaics probably remain undetected, particularly among the phenotypically normal parents of trisomic offsprings) the remainders are translocators (Cassedy S.B. et al, 1977).

G-21 Trisomy

Polani et al (1963) studied the karyotypes of 3 children of mongolism born to unusually young mothers 2 of them had standard Trisomy G-21. Makino et al (1960) in Japan and Hamerton et al (1962) in England reported 10 and 14 mongols, respectively, who were born to young

mothers and found the standard supernumerary G-21 chromosome in all but 2 cases. Lehman et al (1962) who consider 24 years as the upper age limit for a young mother, studied 45 patients with mongolism born to young mothers in Sweden, they found only 1 child had other types of chromosomal aberrations than the standard G-21 trisomy.

The existance of a genetic predisposition to chromosomal errors has been postulated. Thus, predisposition of chromosome errors can be one of the mechanisms leading to G-21 trisomy mongolism among the offspring of parents of all ages and leading to familial concentrations of mongolism as well as other chromosomal anomalies.

The occurance of non-disjunction during the first mitotic division (first cleavage division) of tne zygote can result in the production of two cells, one trisomic for G-21 and the other monosomic for the same. Since autosomal monosomy has not been observed in man, it is supposed that cells monosomic for G-21 die, and the individual developing from the trisomic product of mitotic non-disjunction survives.

Partial G-21 trisomy due to the presence of large pieces of supernumerary G-21 chromosomal material translocated onto other chromosomes, in strict sense, also represents partial trisomy. Cases with supernumerary G-21 chromosome with addition affecting the short arm come under this category. Hall (1963) reported 2 boys with clearcut mongolism.

Translocation

Translocation can occur between large acrocentric chromosome and supernumerary G-21 material (D 13-15 / G-21).

Sporadic translocation

The first chromosomal variation from the standard trisomy for G-21 was reported by Polani et al (1960) who described a typical stigmata of mongolism and only 46 chromosomes with four small acrocentric autosomes instead of the expected five (Carter et al, 1960). There were only five

chromosomes instead of 6 in the D 13-15 group, while an additional submetacentric chromosome was found in the C 6-12 and X-group. This additional chromosome was thought to have originated through translocation between a supernumerary G-21 and a member of D 13-15 group. In this reported case, the parents had normal karyotype. Since these patients are clinically indistinguishable from the regular trisomic type of children with mongolism, it is supposed that genes on the long arm of chromosome G-21 when present in triplicate, are responsible for mongolism. The observation that loss of all the short arm of chromosome G-21 shows no detectable phenotypic effect also supports this assumption (Shaw, 1962).

Sergovich et al (1964) reported a different type of D 13-15 / G-21 translocation. Patient had 46 numbers of chromosomes. The sixth member of group D (large acrocentric) had enlarged short arm with satellite. It was concluded that inserted material originated from long arm of G-21.

Familial translocation

Penrose et al (1960) and Carter et al (1960) simultaneously reported two families where the phenotypically normal mothers of mongoloid children were found to possess 45 chromosomes. Since these mothers were phenotypically normal, most of the genetic material of the acrocentric chromosomes must have been preserved.

Familial transmission of D/G 21 translocation was described by Hamerton et al (1961), Breg et al (1962), German et al (1962), Shaw (1962a, b), Lehman and Forssman (1962), Biesele et al (1962), Forssman and Lehman (1962), Sergovich et al (1962), Hayashi (1963) and Soltan et al (1964). In all of these cases, the mother proved to be the translocation carrier. There is a report of familial transmission of D/G-21 translocation resulting in mongolism in the offspring where the father was the translocation carrier, who transmitted the translocation chromosome to his offspring (Sergovich et al, 1962). The mother had a normal chromosome constitution while the father was found to be a balanced carrier of a

D/G-21 translocation. The paternal grand-father was also a balanced translocation carrier as were at least 6 other members of the family. The patients with D/G-21 translocation represent sporadic cases whose parents have normal karyotype (Soltan et al, 1964). The frequent occurrence of standard G-21 trisomic mongols and D/G-21 translocation mongols in the same families suggests that at least in these familial cases, both types of chromosomal aberration are due to a common underlying cause (Walker et al, 1963; Priest et al, 1963; Soltan et al, 1964; Sergovich et al, 1964). Moorhead et al (1961) reported a standard G-21 trisomic child borned to a mother who was a D/21 translocation carrier. A standard G-21 trisomy mongol and a D/G-21 translocation mongol were also observed in the same sibship born to parents with normal chromosome constitution (Penrose, 1961).

Sergovich et al (1964) found no correlation between the birth of affected children and the age of either parent in cases of D/G-21 translocation. Pfeiffer and Hahler (1964) in mentally retarded mongoloid patients, noted that many of the translocation mongols were born to young mothers as one would expect.

Translocation between two small acrocentric chromosomes (G/G translocation) in mongolism has been described in patients with 46 chromosomes. In such cases, group G is composed of only 3 autosomes while an odd metacentric chromosomes resembling members of F 19-20 group is also present. This can be result of G 21 / G 21 translocation; G 21 / G 22 translocation; G 22 / G 22 translocation or an isochromosome for the long arm of G 21 or G 22. Since the patients with clinical stigmata of mongolism, translocation of the G 22 / G 22 type and isochromosome for the long arm of G-22 apparently can be ruled out. Rest possibilities stand patients with G/G translocation usually have all the clinical stigmata of mongolism. This type of mongolism may occur, both in families and sporadically. Like D / G 21 translocation mongolism, it appears to be more frequently the result of a sporadic event during germ cell maturation rather than an inheritance from a carrier present (Sergovich et al, 1964).

Mosaicism

The most frequently recognised mosaicism involving G-21 chromosome is normal / G-21 trisomy (Aula and associates, 1961; Clarke et al, 1961; 1963; Hayashi et al, 1962; 1963; Lindsten et al, 1962; Blank et al, 1962; Nichols et al, 1962; Richards and Stewart, 1962; Smith et al; 1962; Zellweger and Abbo, 1963; Verresen et al, 1963; Weinstein and Warkany, 1963; Gustavson, 1964). Theoretically, this chromosome constitution can be produced by four ways, all of which involve mitotic non-disjunction or mitotic anaphase lagging. Production of the normal / G-21 trisomic mosaicism from a normal zygote can result, if non-disjunction takes place during the second or subsequent mitotic divisions of the zygote or as a result of non-disjunction during the first cleavage division followed by anaphase lagging in a subsequent division. If a gamete is trisomic for G-21 anaphase lagging during the first cleavage division or during a subsequent division can result in normal / G-21 trisomy mosaicism.

As expected, the clinical picture in cases of mosaicism is quite variable. Probably, the clinical manifestations depend on the "extra dose" of chromosome G-21 present, on the proportion of the cells with supernumerary chromosome material and on the distribution of these cells within the body. Some of the mosaic cases with more than 5% abnormal cells have shown marked mental retardation and other features consistent with the diagnosis of mongolism (Fitzgerald and Lycette, 1961; Richards and Stewart, 1962; Hayashi, 1963; Zellweger and Abbo, 1963; Gustavson, 1964).

In other instances, mosaic patients were reported to have either none or only a few physical features of mongolism and were not mentally retarded (Clarke et al, 1961; Hamerton, 1962; Weinstein and Warkany, 1963; Gustavson, 1964). But in these cases majority of the cells examined had a normal chromosome constitution.

Ford and his colleagues (1959) described a patient who appeared to have both mongolism and Klinefelter's syndrome, having a chromosome number

of 48 with trisomy for chromosome G-21 and an XXY sex chromosome complement.

Genetic counselling in mongolism

The general frequency of mongolism is considered to be about 1.6 / 1000 births. This definitely varies with maternal age, with a remarkable rise after the age of 30 to 35 years. Below the age of 29 years, the risk is 1/3000 between 30 and 34 years, it is 1/600 between 35 and 39 years, it is 1/280 between 40 and 44 years, it is 1/70 between 45 and 49 years, it is 1/40 (Carter and McCarthy, 1951).

The risk of mongolism depends on the nature of the chromosomal anomaly present in the affected child and the parents. If the parents are cytologically normal, the risk of recurrence after the birth of a child with G-21 trisomy is about 1%, regardless of maternal age (Hamerton et al, 1961). The risk is the same if the parents are normal and the child has a D/G-21 or G-21/G translocation.

If the affected child is a G-21 trisomic and either parent shows a G-21 trisomy/normal mosaicism the risk of recurrence is much increased, this will depend on the degree of gonadal involvement.

The majority of cases of mongolism are due to G-21 trisomy, about 2% of all unselected cases are the result of translocation. Among the mongols born to mothers under the age of 26 years, this incidence is higher being about 6%. The highest incidence of translocation mongolism (about 20%) was found in families with a recurrence of Down's syndrome in a sibship. Although the familial transmission of a D/G-21 translocation chromosome has led in many cases to the production of translocation mongols, most of the mongoloid patients with D/G-21 translocation represent sporadic cases whose parents have normal karyotype. If the mother and the patient have a D/G-21 translocation then for a further pregnancy the chances are a little more than 1 in 3 of a normal child; a little more than 1 in 3 of a balanced heterozygote and between 1 in 3

and 1 in 10 of a recurrence of mongolism (Gianelli et al, 1965). If the father is the translocation carrier, the risk of recurrence is low. If either parent is a G-21/G-21 translocation, all children will be affected. If a G/G translocation carrier has had normal children, or if one of the children has a balanced G/G translocation the probable diagnosis. G-21 / G-22 translocation. These have a fairly low risk of recurrence.

A frequent association between acute leukemia and mongolism has been noted at all ages (Merrit and Harris, 1956; Krivit and Good, 1956; Stewart and Hewitt, 1959; Wald et al, 1961). It was found to be 3 to 15 times more common in children with mongolism than in the general population (Stewart et al, 1958).

Mental retardation and multiple congenital anomalies

When a chromosomal anomaly is found in association with multiple malformation, the question arises concerning the relationship between these two aspects. In some cases the chromosomal aberration is present by mere coincidence, while in the other, it has casual relationship (Bartlos et al, 1967). Gross chromosomal aberrations are usually associated with multiple congenital defects. Extra chromatin material in form of extra entire chromosome or of translocations or insertions of portions of chromosome are often compatible with continued life and development. Loss of any one entire autosome is almost always incompatible with life. At least 4 cases of monosomy, with survival for number of years, have now been described (Schaffer J.A. et al, 1977). Mosaicism may exist in individuals who are phenotypically normal or who have all the characteristics associated with the abnormal karyotype or who show same but not all the defects which usually result from that chromosomal anomaly.

Crandell et al (1972) reported chromosomal analysis on 700 consecutive children referred to a child psychiatric clinic showed chromosomal change in 36 (5.1%) of the whole group and in 4.62% of the children with normal intelligence (I.Q. more than 70). 77% of these children were

referred because of emotional problems and 23% because of mental retardation often accompanied by emotional problems. There were 11 sex chromosome aneuploidies, 13 structural anomalies, 12 chromosomal variants. This increased chromosomal changes in children with behavioural disorders emphasise the need for further studies.

The phenotypic abnormalities in cases of translocation are regarded to be result of a genetic imbalance in the form of trisomy for certain genes and monosomy for others.

Francke et al (1977) recommend bone marrow chromosome analysis in new born infant when the following 3 criteria are met with (1) strong clinical suspicion of chromosomal syndrome known to be associated with severe defect in brain development, (2) immediate surgical treatment or assisted ventilation or both are required to sustain life, (3) the parents and physician are prepared to take action depending on the outcome of the procedure.

CHROMOSOMAL ANOMALIES OF AUTOSOMES GROUP : E16-18

This syndrome was not recognised as a separate entity until when Edwards and his associates (1960) described the first patient in detail. Patau et al (1960) described 2 infants with this condition, later on Smith et al (1960) reported 4 more cases. Almost every organ system has been described to be affected in E-18 trisomy syndrome. At least 19 defects were found in more than 50% of patients and no less than 30 other defects have been described in 10 to 50% of the cases (Smith, 1964). Apparent mental retardation and failure to thrive in terms of growth and maturation are constant findings. The common clinical features are general hypertonicity, micrognathia, low set malformed ears, prominent occiput, ventricular septal defect, patent ductus arteriosus, short sternum, small pelvis, renal anomalies and cryptorchidism in males. Flexion of fingers where by index finger tends to overlap the 3rd finger and/or the 5th finger overlaps the 4th. The hand is inclined in either the ulnar or radial direction. Short fifth finger as well as hypoplasia of the finger nails. First metatarsal bone is often short, big toe tends to be held in a dorsi flexed "hammer" position.

In several cases, mothers recalled feeble fetal movements during pregnancy and the placentae that were available for inspection were consistently small (Smith et al, 1962; Hecht, 1963). Anomalous umbilical vessels were noted in several cases (Lenosky and Medovy, 1962; German et al, 1962; Heinrichs and Allen, 1963). The cases were reported with congenital multiple arthrogryposis (Pfeiffer and Huther, 1963), extensive vascular calcification and chondrodystrophia calcificans congenita (Rosenfield et al, 1962), agenesis right lung and enlarged clitoris (Voorhess et al, 1962), optic atrophy and bilateral congenital glaucoma (Townes et al, 1962).

Patients with apparent E-18 trisomy were reported by Edwards et al (1960), Patau et al (1960, 1961), Smith et al (1960, 1962), Uchida et al

(1962a), German et al (1962), Weiss et al (1962), Voorhess et al (1962), Koenig et al (1962), Townes et al (1962, 1963), Rosenfield et al (1962), Gattlieb et al (1962), Holman et al (1963), Heinrichs and Allen (1963), Steinberg and Jackson (1963), Hecht et al (1963b), Lejeune et al (1963), Pfeiffer and Hunter (1963), Tolksdorf et al (1963), Sanchez Cascos et al (1963), Lewis (1964), Turner et al (1964) and Warkany et al (1964).

Double trisomy

Gangnon et al (1961) reported a child with 48 chromosomes, two supernumerary chromosomes resembling members of group E-18 and G 21-22 respectively.

Uchida et al (1962b) found female child with XXX chromosome with a trisomy for chromosome E-18. Koulischer et al (1963) reported E-18 trisomy / normal mosaicism in a patient manifested a complete phenotypic expression of the E-18 trisomy syndrome. Extra E-18 chromosome was found in 64% of cells of the cultural skin cells. Weiss et al (1962) reported similar patient.

Warkany et al (1964) reported an 8½ year old girl with 47 chromosomes with E-18 trisomy in 70% of the cells examined and 46 chromosomes in 20% of cells. Other findings include advanced maternal age at birth, intrauterine growth retardation, severe psychomotor retardation, failure to thrive, possible congenital heart disease, premature fusion of sternal sutures, renal anomaly and hammer toe; also showed picture of cerebral palsy. Zaleski (1963) described two sibs with similar phenotypic and chromosomal aberrations found to have trisomy for the short arm of one of the members of E-16-18 chromosome group.

Wang et al (1962) described a male child who had 46 chromosomes including an A-3 chromosomes longer than its homologue, this was thought to represent a translocation piece of E-18 on to A-3.

Gangman et al (1963) reported a female child with 46, XX chromosome including an abnormally long B-4 chromosome translocation of long arm of E-18 and B-4.

CHROMOSOMAL ANOMALIES OF GROUP D13-15

The D-1 trisomy syndrome (Patau's syndrome). The first probable report of this syndrome was that of Bartholin in 1657 (Warburg and Mikkelsen, 1963). Kundrat (1882) reported some pattern of congenital anomalies under the heading of "Prosencephaly". Patau and his colleagues (1960) set the etiology and further definition of this entity. Therman et al (1961) used the suffix 1 (D_1) to denote the autosome found in triplicate in the first recognised D-trisomy syndrome.

This syndrome has been described only in the paediatric group. Smith (1964) evaluated 10 infants with the D_1-trisomy syndrome in the State of Wisconsin; at the time of report only 2 children were living at the ages of 2½ and 5 years. Both had grown at a normal rate during early infancy, but have subsequently failed to thrive in terms of both weight and length. They were severely mentally retarded, unable to sit, and continued to have myoclonic jerks and apnoeic spells. 69% of cases died by the age of 3 months.

EMBRYOLOGY : NORMAL AND ABNORMAL SEX DEVELOPMENT

In order to understand the varied anatomical findings in intersexual states, one has to be familiar with the embryology of normal sex development.

It is interesting to note that all three germ layers participate in the development of the urogenital system. The mesodermal components arise from the wall of the coelom in the intermediate cell mass. These include following :

1. The nephric system
2. The Wolffian (mesonephric) duct
3. The Mullerian (paramesonephric) duct
4. The gonads.

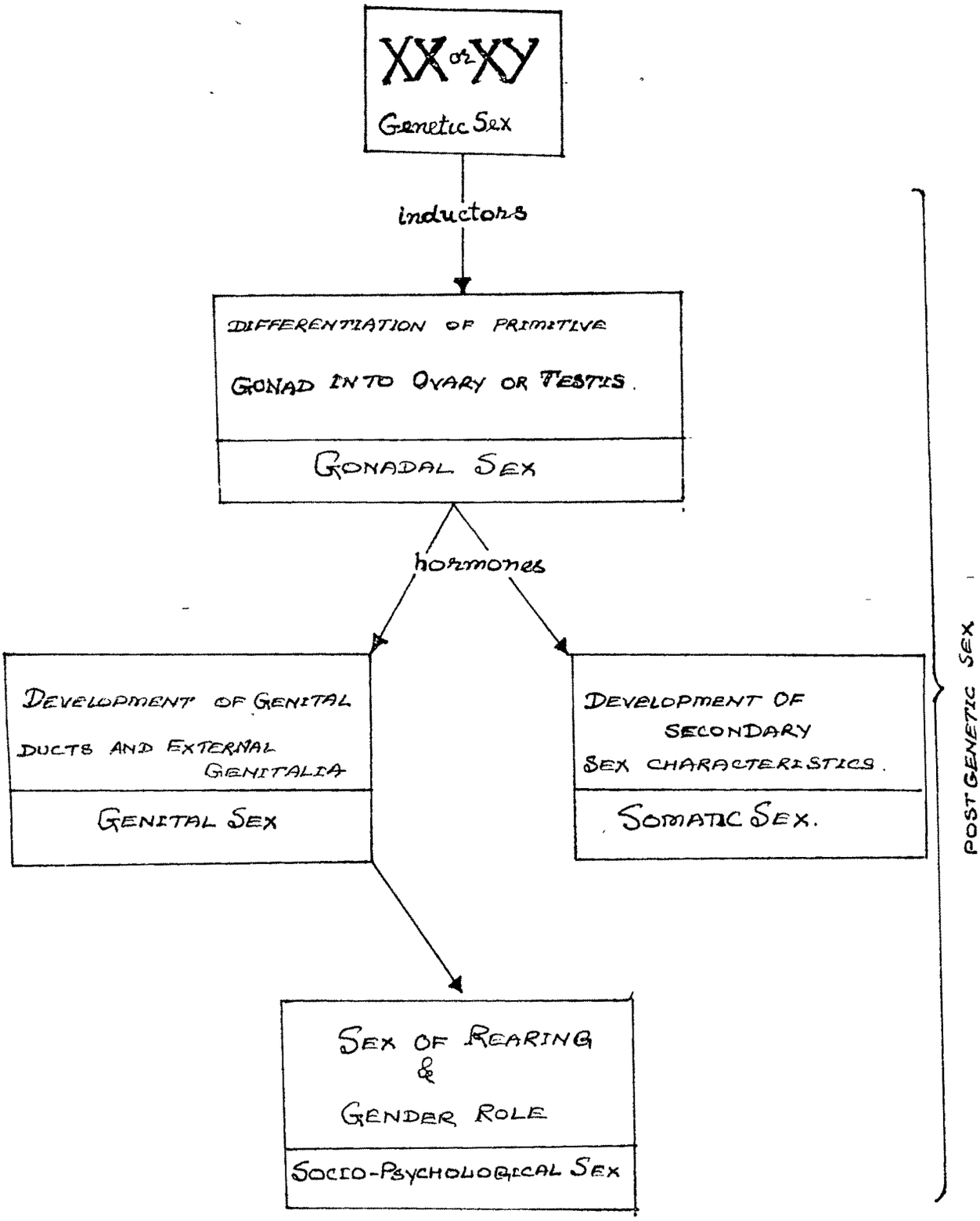
A common endodermal cloaca, which is the dilated terminal end of the hind gut, participates in the development of this system. The ectoderm contributes to the development of the external genitalia.

Three sequential processes are involved for normal sexual development.

The first step is establishment of genetic sex. The differentiation of sex is determined primarily by the sex chromosomal constitution present at the time of fertilization. The presence of Y-chromosomes, present only in man, is responsible for initiating embryonic differentiation of the gonads.

The second step involves the translation of genetic sex into gonadal sex under the influence of sex chromosomes, the undifferentiated gonads differentiate into testis in the presence of Y-chromosomes in the male or into the ovary in the presence of XX-chromosomes and in absence of Y chromosome in the female.

Third step considered to be a final step where the differentiated gonad (male or female) influence the development of phenotypic sex (internal



SCHEME OF SEXUAL DIFFERENTIAN IN MAN [MEDICAL CYTOGNETICS BARTALOS M; (1967)]

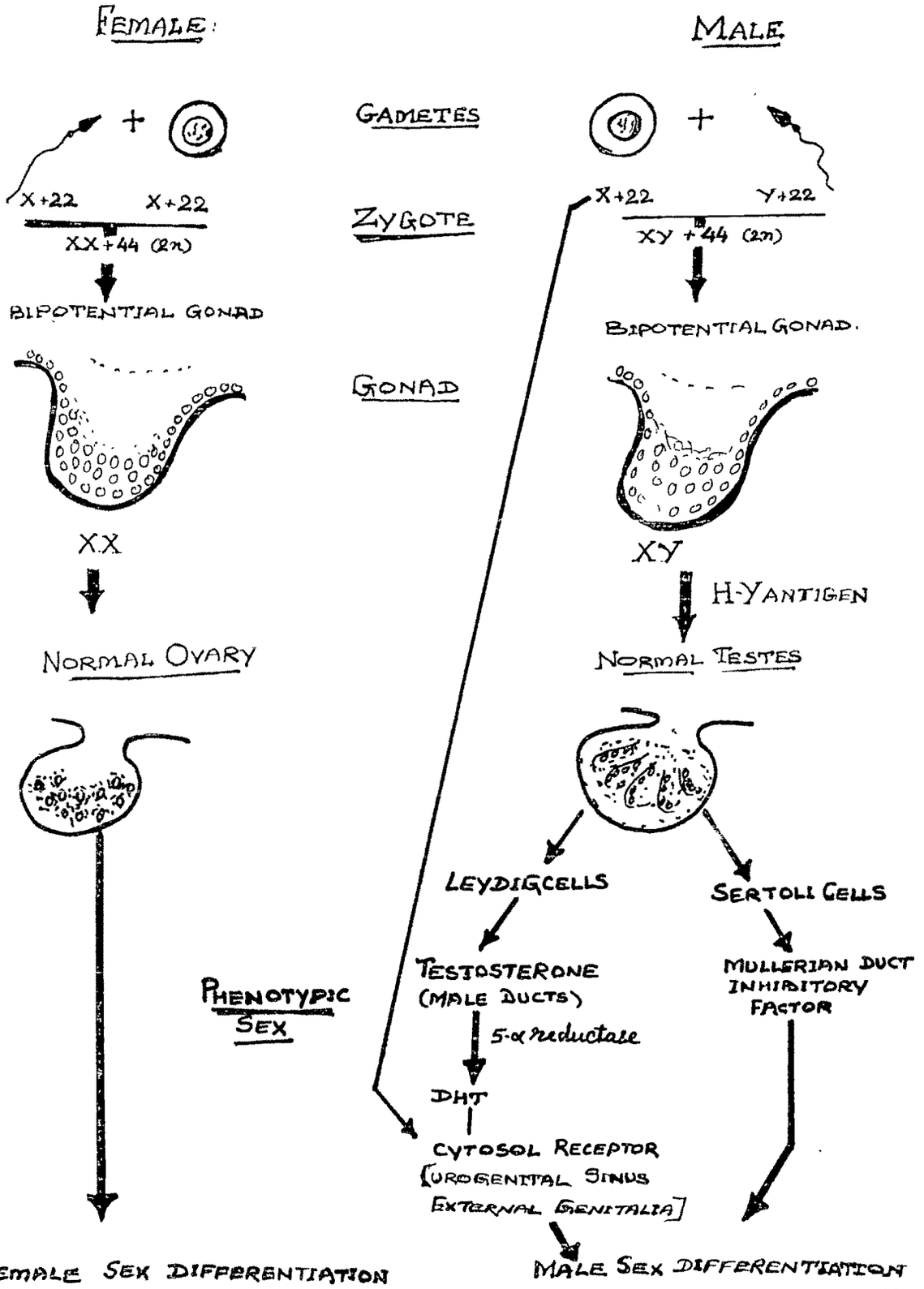
FIG: 1

genitalia). At puberty, the development of the sex specific secondary sex characteristics, reinforce and provide more visible phenotypic manifestation of this sexual dimorphism and the behavioural and functional characteristics are determined.

Thus, the principal stages leading to normal sex development and differentiation of gonads are development of genital ducts, differentiation of the genitalia, development of secondary sex characteristics and fertility. Either any one or all of these processes may be disturbed in a genetic disorder.

The first event of the sex development is the migration of a primordial germ cells from the wall of the yolk sac to the genital ridges at the age of about 6 weeks of intrauterine life. The thickening in the ridge indicates the position of the mesonephric (Wolffian) and uterine (Mullerian) as well as nephric systems. Sex chromosomes constitute influence the differentiation of goands. In the presence of androgen secreting Leydig cells the mesonephric duct normally differentiates to form the typical male duct system. At the same time, the Sertoli cells produce a second factor, the Mullerian inhibiting factor, which causes regression of the uterine duct system. If an ovary is present, or if the gonads remain undifferentiated, usually a typical female duct system develops and the mesonephric system regresses (Fig.1).

In the early development, the embryo consists of a genital tubercle, paired labio-scrotal swellings and paired urethral folds, as an external genitalia. The genital tubercle can form either penis or clitoris. In the male, under the influence of androgens, secreted by fetal Leydig cells, the labio-scrotal folds fuse to form the scrotum and the urethral folds fuse to form the urethra including its penile portion, while genital tubercle takes on a male (penile) configuration. In the absence of a differentiated gonad or in the presence of an ovary, the labio-scrotal fold remains separate, to form the labia-majora, urethral folds remain unfused to form the labia minora and the genital tubercle assumes the female (clitoral) configuration.



Drawing representing the Human Sex differentiation:
Anomalies of Sex may arise because of disturbance of Intrinsic or Extrinsic factors

FIG: 2

Differentiation of male external genitalia is an active process, requiring the presence of androgen. Female external genital development however, so passive and may occur in the absence of any gonadal secretions. In female development minimal evaluation of fetal structures occurs. In males, there is elongation of phallus, midline fusion of paired fold primordia and posterior migration of developing scrotal sacs.

Thus, sex identification may be described in terms of the following characters :

1. Chromosomal constitution
2. Gonadal structure
3. Morphology of genital ducts
4. Morphology of external genitalia
5. Hormonal status
6. Sex of rearing
7. Gender role.

As it is evident that first five of these characteristics are organic, while the last two are psychologic. The last six characteristics may be collectively spoken of as post-genetic sex (Fig.2).

Androgen of any source causes differentiation of the bipotential external genitalia along male lines. These androgens may originate in fetal testis, fetal adrenals (as in congenital verilizing adrenal hyperplasia) or from the maternal side of the placenta. If no androgens are present, the pathway of development is that of females. Thus, in cases of gonadal dysgenesis or peripheral unresponsiveness to androgens (testicular feminization) normal female genital development occurs.

If external development is incomplete in either direction, genitalia remain ambiguous, resulting in inter-sexuality. Partial male genital development may result in hypospadias, a bifid scrotum and a small penis. Complete failure results in female genitalia.

Virilization of a female fetus may result in clitoral hypertrophy, labial fusion and in extreme cases, a penile urethra and fused scrotal folds (female pseudohermaphroditism). As a general embryonic rule, presence of 2 sex chromosomes is necessary for the gonads to develop fully. If only one normal sex chromosome is present, the gonad will not develop. Chromosomal aberrations or single gene mutation may cause ambiguous development of external genitalia.

Primary amenorrhoea is a condition which may occur in many different disorders. Failure of menstruation in a girl by the age of 18 years is usually considered as primary amenorrhoea. Delayed menarche is considered in female between the age of 14-17 years who have not had their first menstrual period. The etiology of this condition may be due to more or less severe abnormalities of structure and function of the urogenital system.

Patients with primary amenorrhoea can be divided into three groups : those with an abnormal chromosome constitution like Turner's syndrome or those with sex chromosome in a disagreement with the phenotype like testicular feminization and those who have a normal 46, XX chromosome constitution with Mullerian or ovarian agenesis.

This shows that the differentiation is complicated sequence. Sex determination is very critical and it may go wrong at almost any point during the process of meiosis at time of zygote formation or during post-zygote cell division which may lead to chromosomal anomalies. These abnormal anomalies may be spontaneous events or may be induced (by chemicals, by radiation therapy, environment etc.) during these steps. As a result an individual develops with additional or deleted chromosome; structurally altered chromosome or with two or more types of cell lines.

SEX CHROMOSOMES

Evolution of genetic sex determination

According to Wilschi (1959), the origin of genetic sex determination in

tetrapod vertebrates dates back almost to the Jurassic period, about 150 million years ago.

Sex determination in vertebrates, which is dependent upon an established chromosomal basis, probably arose from a state of hermaphroditism or from a non-genetically determined bisexuality. Genes causing sexual differentiation may have originated through series of mutations as they were originally scattered through out the whole chromosome set. That stage was followed by the accumulation of mutant genes which favoured one sex in one chromosome and the other sex in another chromosome. Such a stage can still be observed among the majority of the fishes (Wilschi and Opitz, 1963).

The following stage consisted of the isolation of the sex determining segments of those chromosomes by a reduction of crossing over, thus insuring the preservation of gene combinations and favouring sexual separation (Swanson, 1957). Such a stage can be observed in amphibia, where the sex chromosomes are hard to distinguish from the autosomes.

Birds and mammals, on the other hand, possess two clearly different kinds of chromosomes plus two 'X' chromosomes. Thus, normal human females have 44 chromosomes plus two 'X' chromosomes. While, normal males have 44 autosomes plus one 'X' and one 'Y' chromosomes.

The 'X' chromosome was first observed during the spermatogenesis of the red bug Pyrrhocoris apterus by Henking in 1891. Its sex determining role, however, was not pointed out until 13 years later (McCling, 1902). In man, it is a medium-sized submetacentric chromosome which belongs to the C_{6-12} and X group of chromosomes (Denver system of nomenclature). It is approximately the second largest chromosome in this group and its centromere is more medially placed than any of the larger members of this group. In the somatic cells, one of the two 'X' chromosomes (the sex chromatin forming one) replicates quite late during DNA synthesis.

The 'Y' chromosome is a small acrocentric, one which belongs to group G_{21-22} and Y. It is usually the largest of this group, its long arms tend to be close together and it has no satellites. It replicates later than any of the small acrocentric chromosomes, a finding which is compatible with the heterochromatic nature of this chromosome.

The difference in size between the X and Y chromosomes has been estimated to give the 4% more genetic material than the normal male. This is based on the fact that the 'X' chromosome is about 4 times as long as the Y chromosome (Ford, 1963).

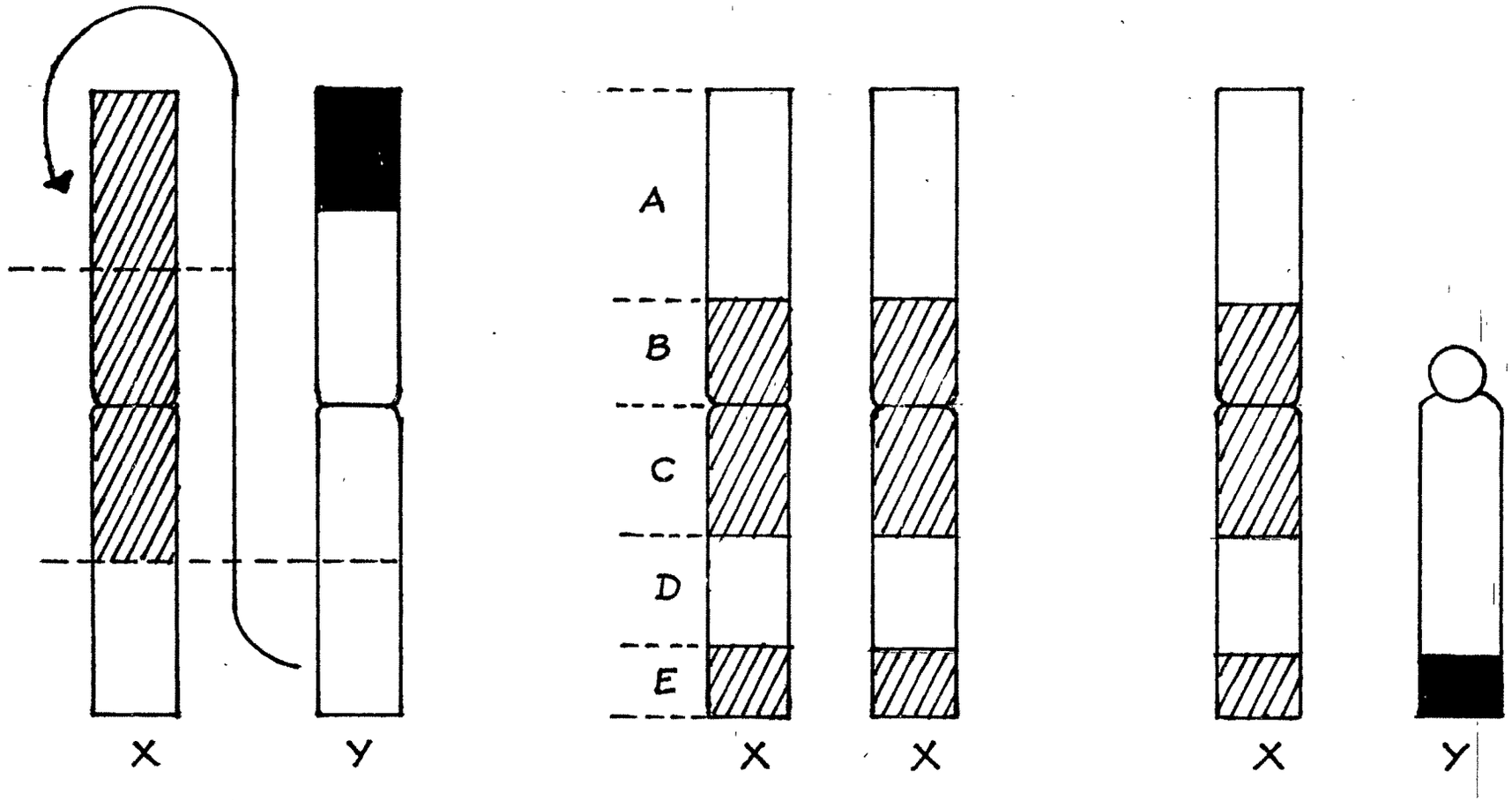
During spermatogenesis sex chromosomes form a single bivalent, where the long arm of the Y chromosome and the short arm of the 'X' chromosomes are attached by end to end synapsis. This is suggestive of the presence of very small pairing segments (Ford, 1963).

These two chromosomes are characterized by early separation and positive heteropyknosis in the spermatocytes (Roosen-Rung, 1962).

Eberle (1964) proposed that the X and Y chromosomes are genetically inactive during spermatogenesis in man, giving an equal fertilizing chance to the 'X' bearing and 'Y' bearing sperms as well as to sperms which are lacking the sex chromosome. He added that end to end association of the 'X' and 'Y' chromosomes may impart a survival advantage to a given species by allowing 'X' and 'Y' chromosomes to segregate without chromatid exchange and crossing over, ensuring the differentiation of the sexes. This is believed to be an older evolutionary development than the chiasmatic association of the autosomes during meiosis.

Evolutionary Theories of Gonosomal Morphology

It is interesting to note that theories of the phylogenetic evolution of gonosomal morphology as described by Lyon (1974) and Hoo (1975) contribute both logical and "historical" ideology to some of the hypothesis mentioned above. According to these theories of mammalian



SEX CHROMOSOMES OF EARLY VERTEBRATES.

HUMAN FEMALE SEX CHROMOSOMES.

HUMAN MALE SEX CHROMOSOMES.

FIG.: 3 - HYPOTHESIS CONCERNING THE MORPHOLOGICAL EVOLUTION OF SEX. (ACCORDING TO HOO - 1979).

evolution, progressive modifications of originally similar gonosomes led to the development of a pair of dissimilar chromosomes. So that eventually only the so-called B and C fragments (Fig.3) preserved their intrapair homology.

The subsequent transfer of segment "A" from the 'Y' to the 'X' chromosome would, then, result in the duplication of many X-linked genes. According to the theories of Lyon (1974), the inactivation of one of the two X chromosomes of the females or at least of segment A and D, would then be necessary to avoid potentially harmful repetitiveness.

It is conceivable that the B fragment of Hoo's model and the chromosomal segment postulated by Therman (1976) to be the remaining active 'b' segment of both 'X' chromosomes are analogous. These explanations would be consistent with the finding that gonadal function is preserved in patient with Xp terminal deletions (that is, partial deletion of the A fragment) (Hoo, 1979); one might have a deletion of that fragment without consequence, if much of the same genetic material is also located on the D fragment, which remains.

One might wonder, why a similar mechanism would not correct the short stature and other extra-gonadal stigmata associated with Turner's syndrome. A potential explanation would take into account the fact that certain Turnerian features such as short stature are in comparison with the rather specific anomaly of gonadal dysgenesis of a more quantitative nature due to their multifactorial determination. This fact would probably render minor the influence of a particular but of genetic material (such as the postulated D fragment).

However, some inconsistencies also exist between these evolutionary theories and true clinical observation. For example, Hoo's model does not account for the presence of a "critical region" as postulated by Sarto et al (1973) and supported by their patient data; in addition it would not predict the (end to end ?) pairing images actually observed at meiosis of the human sexual bivalent (Luciani, 1970a,b).

During agenesis, the two X chromosomes are genetically active (Park, 1957) they are isopyknotic and undergo synapsis with crossing over (Ohno et al, 1960).

The fact that the 'X' and 'Y' chromosomes are morphologically different suggest that they carry different genes. This has been demonstrated by family studies which showed the presence of specific genes on the 'X' chromosome with no counter part on the 'Y' chromosome. Such genes are called sex-linked or more correctly X-linked genes. The 'Y' chromosome, on the other hand, may contain genes which do not have a counter part on the 'X' chromosome and these are referred to as holandric genes. Animal studies have shown that the 'X' and 'Y' chromosomes have corresponding parts (homologous parts) which contain similar genes called incompletely sex-linked genes. In man, this is still a questionable point.

The 'X' chromosome carries a number of genes which have been identified in association with various pathological traits.

With the help of techniques like somatic cell hybridization pedigree analysis and cytogenetic banding methods for chromosome identification, the loci for hypoxanthine guanine phosphoribosyltransferase, G6PD, phosphoglycerate kinase, L-galactosidase, colour blindness, hemophilia A, adrenoleukodystrophy and a "fragile sites" associated with micro orchidism and mental retardation, among others have been assigned to the long arm of the X-chromosome (Keats, 1983; Chapelle de la et al, 1979).

The 'Y' chromosome in man seems to carry little specific genetic information besides its role in sex determination. The trait "hairy ears" (hypertrichosis of the pinna of the ear) may be determined by a holandric gene (Sarkar et al, 1961; Gates and Bhadury, 1961; Dronamraju, 1960; Gates et al, 1962; Dronamraju and Holdane, 1962).

Keratoma dissipatum hereditarium palmare et plantare may also be a Y-linked trait (Brauer, 1913; Vagel, 1961).

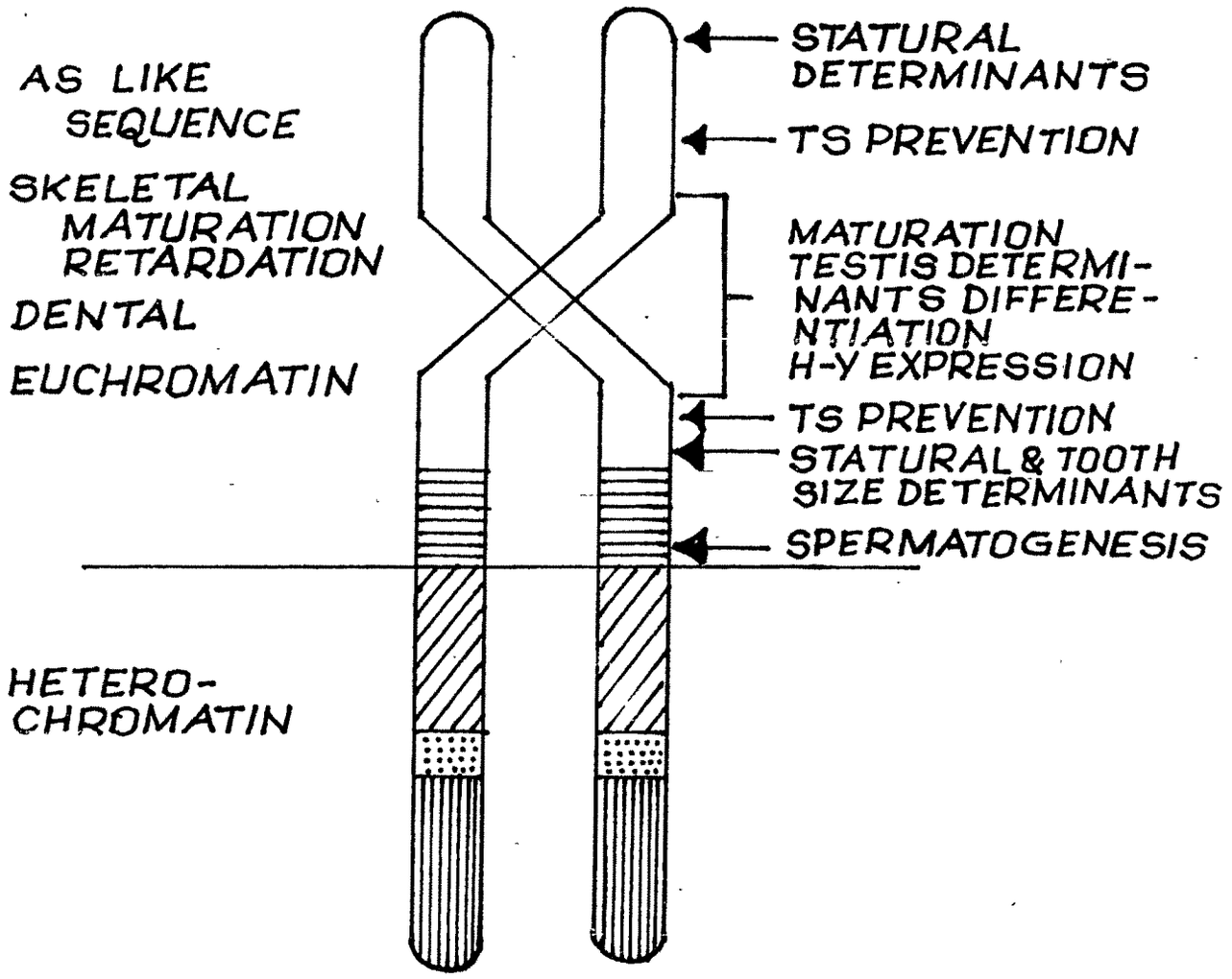


FIG.:4 Y-CHROMOSOME

There are indications that genes on the 'Y' chromosomes are responsible for delayed skeletal maturation and other signs of physical maturity in the male. This action of the 'Y' chromosome probably begins at about the 7th week of intrauterine life (Janner et al, 1959).

Human Y-Chromosome : Its biological functions and structure (Fig.4)

The mammalian X and Y chromosomes evolved from a homologous pair of chromosomes that differ only at the locus regulation sex determination (Ohno, 1979). It was believed that the Y-chromosome was inert and that male determinants were carried on the autosomes. After the advant of human chromosomal analysis, the findings of an 47, XXY pattern in patients with Klinefelter's syndrome established that Y-chromosome carries male-determining genes that can induce testicular development even in the presence of two or more X-chromosomes. This conclusion is supported by the discovery of H-Y antigen, the positive morphologic factor for the testis and documentation of a role of a Y-linked genes in its expression (Ohno, 1979; Wachtel, 1983; Wachtel and Ohno, 1979). Even in the presence of poly 'X' as in 48, XXXY individuals, Y-chromosome leads to testicular differentiation, whereas, absence of Y in 45,X individuals, the testicular differentiation does not occur. Y is not only differentiates premature gonads in to male gonads but is also essential for spermatogenesis.

The size of the human Y-chromosome varies considerably from normal to three-fold in length in normal men (Grumbach and Conte, 1985; Bender and Gooch, 1961; Bishop, Blank and Hunter, 1962; de la Chapelle et al, 1979). The morphology of the Y is inherited from one generation to other, and is relatively constant in male relatives and exhibits racial variation. Most of the variation is limited to the length of the long arm and its distal heterochromatic, brilliantly fluorescent segment in Q-banding preparations. This polymorphism in size of the fluorescent portion, as well as loss of the part of the distal non-fluorescent portion of the long arm is consistent findings with normal sex differentiation and not associated with recognised phenotypic effects. The large segment of

the long arm of the Y is probably not engaged in gene transcription (Buhler, 1980). The long arm of the Y contains repetitive sequence of DNA that are both specific and non-specific to the Y chromosome.

The major function of the Y-chromosome is to direct the bipotential embryonic gonad to differentiate into a testis and to ensure spermatogenesis. Only a few other genes loci have been assigned to the Y (Buhler, 1980). Perhaps a regulatory or a structural gene locus for H-Y antigen (the testis organising factor), possibly in multiple or repetitive copies is situated on the short arm of the Y close to the centromere or possibly on the pericentric region of the long arm (Koo et al, 1977; Davis, 1981). The relationship of the Y-specific DNA to the gene(s) encoding H-Y antigen is unclear at present, although the sex-specific DNA sequence may specify, at least in part, the H-Y plasma membrane antigen (Epplen et al, 1982; Ohno, Epplen and Celline, 1984).

Improvement of cytogenetic techniques and advances in immunology and molecular genetics have shown that only a very small part of the short arm of Y-chromosome is required for gonadal differentiation into a testis. The product of this gene, the testis determining factor (TDF) has not been characterised. It was thought to be identical to the H-Y antigen. Examination of various sex chromosomal rearrangements have shown that TDF locus is different from the H-Y locus. TDF may be lying near the distal tip of Yp (Goodfellow, 1987) and H-Y lying in the centromeric region or on the proximal region of the long arm (Ferguson-Smith, Affara and Magenis, 1987).

In the 7th week of development of embryo the presence of the TDF gene appears to launch a process that leads to male sexual development, without it, the fetus would be female (Page et al, 1987).

The Y contains loci homologous to those on the short arm of the X and recombination can occur between these homologous sequences (Koller and Darlington, 1934; Keitges et al, 1985; Cooks, Brown and Rappold, 1985; Simmler et al, 1985; Herbers et al, 1983). The presence of a Y-

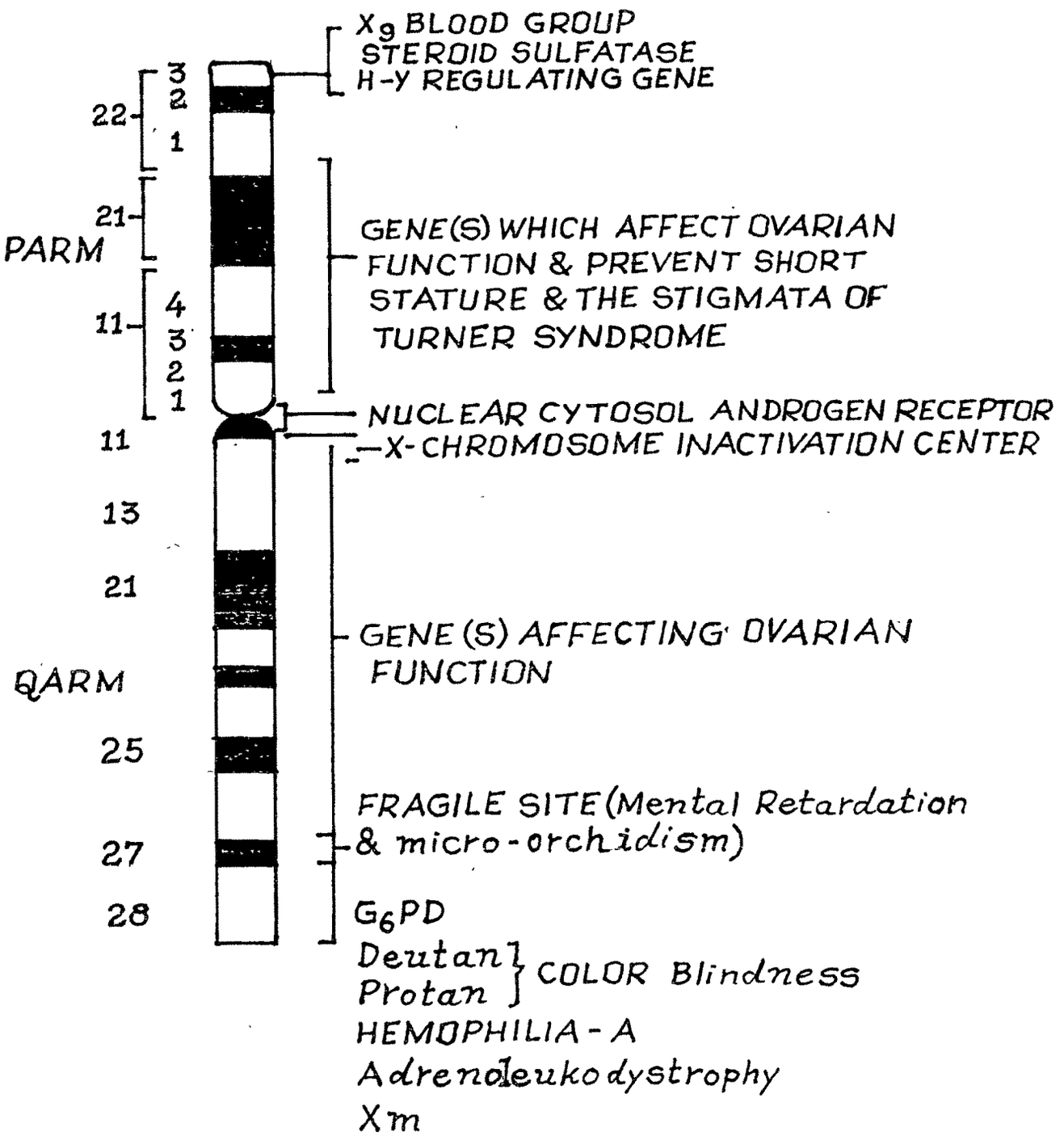


FIG.: 5 X-CHROMOSOME

[ELEMENTS OF MEDICAL GENETICS
A.E.H. EMERY, 1988]

chromosome with a normal X prevents the short stature and somatic abnormalities found in Turner syndrome (Morishima, Grambach, 1968; Grumbach, 1979). This hypothesis has been substantiated by DNA hybridization techniques (Page et al, 1982).

Boczkowski (1971) postulated a testis differentiating factor (TDF) and a testis inhibiting factor (TIF) on the X-chromosome. In the male the TIF is inhibited by a repressor situated on the Y-chromosome, so that testis develop. In the female, TIF inhibits the TDF. An ovary differentiating factor (ODF), also located on the X causes development of ovaries in the female.

According to Siebers et al (1973) maturation of testis is said to be governed by factors on Yp. These authors postulate that the more distally a Yp deletion takes place, the farther the maturation of testis will proceed and the better their function they will be. The slightest degree of maturation inhibition is reduced fertility as described by McIlee et al (1966) in case of long arm dicentric Y [dic (Yq)].

Y-fluorescent body

The Y-brightly fluorescent body (Y-chromosome) is observed in human male metaphases when it is stained with quinacrine mustard fluorescent dye; and same Y-body can also be seen at interphase nuclei of the male, including lymphocytes, hair root sheath cells, polymorphonuclear leukocytes, buccal mucosal cells, and cells grown in culture (Pearson, 1972). The heterochromatine part of distal end of the long arm of the Y-chromosome is fluorescent. In 46,XY male a single Y-body sometimes bipartite in structure is present. While two Y-bodies are detectable in 47,XYY and 48,XXYY males. The body is present in slightly less than 50% of mature sperms (Pearson, Bobrow and Vosa, 1970).

Normal functions of the X-chromosomes (Fig.5)

The functioning of X-chromosomes are more complex. Genes located on the X-chromosomes have a critical influence on sex determination in both the

female and the male and on the differentiation of the somatic sex structures in the male. In addition, over 100 gene loci unrelated to sex development are X-linked (Mukusick, 1983).

According to Wachtel (1983) and Naftolin and Butz (1981) the regulatory or a structural gene, for H-Y antigen is present on the short arm of X-chromosome. Presence of two X-chromosomes are required in humans for normal ovarian differentiation and follicular maturation studies of patients with 45,X individuals showed bilateral streak gonads, various types of deletions of one of the long and short arms are involved in ovarian function (Grumbach, 1968; Ferguson-Smith, 1965). Over and above, the gene that codes for the cytosolic receptor, a major factor in male differentiation is located on the X-chromosome (Mayer et al, 1975).

When both X-chromosomes are normal, as in normal female, with active genes; it present short stature and many of the somatic abnormalities found in the syndrome of gonadal dysgenesis. They are found mainly to be located on the short arm of the X. Similar genetic loci have been reported on the short arm of the Y. The genes for steroid sulphatase, the Xga red cell antigen, and the locus influencing the expression of H-Y antigen have also been localized on the distal region of the short arm of the X-chromosome (Xp 22.2 $\longrightarrow$ pter) (Keats, 1983; Chapella de la, Miller, 1979; Mohandas et al, 1979). These genes remain active on the 'inactive' X-chromosome. Moreover, this segment of the X-chromosome pairs with short arm of the Y-chromosome to form a synaptonemal complex at early pachytene (Solari, 1980). The pairing of the X- and Y-chromosome at zygotene may be a consequence of genetic homology (Poland, 1980; Burgoyne, 1982). Indeed, the distal short arms of 'X' and 'Y' chromosome exhibit similar early replication patterns (Muller and Schempp, 1982).

These observations suggest a degree of base sequence homology in the regions of the short arms of the X and Y that pair during meiosis. Apart from this genetic homology, there are large number of unpaired genes on the X-chromosome, absent in the Y, are responsible for a wide variety

of sex-linked traits. With the help of advancement in technique like somatic cells hybridization, pedigree analysis and cytogenetic banding methods for chromosome identification, the loci for hypoxanthine guanine phosphoribosiltransferases, G6PD, phosphoglycerate kinase, L-galactosidase, colour blindness, hemophilia A, adrenoleukodystrophy and a "fragile sites" associated with archidism and mental retardation among others, have been assigned to the long arm of the X-chromosome (Keats, 1983; Chapelle de la and Miller, 1979) (Fig.5).

Y-chromosome is one of the smallest among human chromosome and is mainly concerned with the formation of testis. The X-chromosome is the eighth longest and contains genetic codes for functions involving every system in the body. Since females have double the amount of this genetic material in their cells as do males, the biological differences between the sexes could have been for greater than is actually the case. This paradox is very well explained by Barr or sex chromatin body in the somatic cells of females.

Barr Body - (X-chromatin)

In 1949, Barr and Bertram described a chromatin mass at the periphery of the nucleus in resting ganglion cells of female but not of male cats. This characteristic of the female sex is present in the peripheral cells of most mammalian species and has been used as a means of accessing the number of X-chromosomes in subjects with errors of sex differentiation. This X-chromatin body is present to the inner surface of the nuclear membrane. Buccal mucosal cells are the most commonly used preparations for determining the X-chromatin pattern. A second sex specific internuclear drumstick body was reported in polymorphonuclear leucocytes by Davidson et al (1954).

Abnormalities in size and shape of the X-chromatin body can often be correlated with the structural abnormalities of the X-chromosome. In female with one normal X and one X with a deleted arm (XXp) shows small X-chromatin. A large X chromatin body is associated with a long

arm isochromosome of X-chromosome (Xq). When a structurally abnormal X is present, it is the aberrant X-chromosome that replicates late and gives rise to the X-chromatin.

The sex-chromatin results from one of the two X-chromosomes in the interphase nuclei of the female somatic cells. It is also observed that the X-chromosome that gives rise to X-chromatin completes its DNA synthesis later than does any other chromosome in the cell, and the maximal number of X-chromatin bodies in a single diploid nucleus is equal to the number of late replicating X-chromosomes (Grumbach et al, 1963; Morishima et al, 1962). These observations and the genetic studies of Beutler and colleagues, Lyon and others led to the concept that only one X-chromosome per cell is genetically inactive for many of its functions (Beutler et al, 1962; Lyon, 1972; Lyon, 1983).

The change of one of the X-chromosome in to heterochromatin in each female cell appears to be induced during the late blastocyst stage, between the 12th and 18th day in the human embryo. Beyond the stage of oogonia, the female germ cells are the only cells known to be exempted from heterochromatin formation as both X-chromosomes are necessary for normal ovarian differentiation. Both X-chromosomes in oocytes are active and code for the X-linked genes G6PD and hypoxanthine guanine phosphoribosiltransferase (Epstein, 1972; Gartler et al, 1976). In all other cells, either maternally or paternally derived X-chromosome is inactive. Once this inactivation is confirmed same X-chromosome is transmitted to all descendents of that cell. This control system serves as a mechanism of dosage compensation by which each female somatic cells function virtually as if it had only one genetically active X-chromosome (Lyon, 1983). The female, therefore, in effect has no more active genetic material than does the male. This hypothesis is known as "inactive X-theory" the "Lyon hypothesis".

Individual with 46,X and 47,XXY constitutions, for example, have abnormalities both in their sexual development and in somatic features

unrelated to sex (Therman et al, 1980). There are two obvious possibilities :

01. The abnormalities may be initiated at an early embryonic stage before inactivation takes place.
02. The original X-inactivation may not be regular or random in all cells with abnormal X-chromosome constitutions. Cells with abnormal X-chromosome numbers being genetically unbalanced, would give rise to abnormal phenotypes.

The red cell antigen Xq and the steroid sulphatase loci escape inactivation and are expressed in both X-chromosome in females. These genes are present on the distal part of the short arm of the X (Mohandas and Sapiro, 1983). This suggests that in normal individuals loci on both the heteropyknotic X and on the Y-chromosome are paired with a locus on the active X and express dosage effect.

GENES AND ORGANOGENESIS

(1) Testicular development

Fertilization of a normal ovum by an X- or Y- bearing sperm leads to the establishment of genetic sex. The differentiation of primordial gonad under the influence of genetic sex into a testis or an ovary are not very well understood. The pericentric region of the Y-chromosome contains genes that act in a dominant fashion and lead to differentiation of the bipotential gonad into testis.

The close relation between the presence of a Y-chromosome and a male phenotype suggests that there are gene(s) located on the Y that determines sex differentiation in mammals. Such gene(s) are likely to be involved in early events of testicular development and may trigger the differentiation of the male gonad from an analogue that is morphologically indistinguishable in the prospective male and female gonadal differentiation. Sertoli cells are the first cells in testis to be distinguished morphologically (Jost et al, 1981). Y-chromosomal sex determining gene(s) may be involved in the differentiation of these cells. However, nothing is known about the nature of these genes. It is now well known that the human Y-chromosome consists of a euchromatic and of a heterochromatic portion (Gaspersson et al, 1971; Schempp et al, 1982). The former is present on entire short arm (Yp) and proximal long arm. The occurance of Y-chromosomes lacking the heterochromatic portion of Yq in normal fertile males (Verma et al, 1978; Davis, 1981; Goodfellow et al, 1985a) suggests that this portion does not contain genes required for male development and function. Such genes appear to be confined to the euchromatic portion of the Y-chromosome (Buhler, 1980). This portion may comprise 30% to 40% of the Y-chromosomal DNA content (Cooke et al, 1983).

As stated by Wachtel and Ohno, regarding the hypothesis for the organogenesis of testis, that the pericentric region of the Y-chromosome

contains a locus that either codes for the plasma membranes H-Y antigen or regulates it expression. The H-Y antigen is disseminated by cells in gonadal blastoma (possibly Sertoli cell precursor) (Zenzes et al, 1978) that binds to gonad specific H-Y receptors and induces differentiation of the primitive gonad in to testis. The embryonic gonad has an inherent tendency to form an ovary in the absence of H-Y antigen or its specific gonad receptors. The testicular organising function of H-Y antigen is indirect and circumstantial. But some experimental data provides direct support for the hypothesis (Ohno and associates). Zenzes and colleagues have reported cell dissociation and reaggregation experiments in new born rat and mouse gonads. Ohno et al (1979) experimented on bovine fetal ovaries and Zenzes et al (1978) worked on cell suspension of new born rat reorganised to form siminiferous tubules like structure when exposed to H-Y antigen. Similar studies with human XX undifferentiated embryonic gonads also resulted in testicular organogenesis. This experiments provide the most direct evidence for the testicular organising function of H-Y antigen. The capacity of H-Y antigen to induce the indifferent embryonic XX or XY gonad primordium to differentiation into testis appears to be a consequence of the presence of gonad-specific H-Y receptors in both sexes.

Recent studies indicate that a very small portion of the short arm of the Y-chromosome triggers the undifferentiated gonads into a testis. The product of this gene is known as testicular differentiating factor (TDF). The presence of TDF on the Y-chromosome is now accepted. Many researchers (Chapelle, 1987; Goodfellow, 1987; Ferguson-Smith, 1987) tried to find out the nature or mode of function of TDF by observing XX males, XY females; XX true hermaphrodites; ultimately the mode of testicular organogenesis.

Distal part of Yp contains TDF locus (Goodfellow, 1987; Ferguson-Smith et al, 1987) and H-Y locus is in the centrometric region or on the proximal region of the Y-long arm. Ferguson-Smith (1966; 1987) have shown that loss of Yp sequences (including TDF) in female patients with XY gonadal

dysgenesis may result from X-Y interchange in a manner analogous to XX males.

(2) Ovarian development

For the differentiation of primary gonad into a normal ovary both intact X-chromosomes are required (Grumbach et al, 1958). In 45,X individuals as well as those with deletion of the short arm (Xp) or long arm (Xq) of the X-chromosome, ovarian development commences in utero, but oocytes usually do not survive meiosis, and follicular development fails to occur or is defective. This leads into loss of germ cells, oocyte degeneration, and secondarily, gonadal X-chromosomes remain to be active from the beginning of meiosis to ovulation (Epstein, 1983). It is concluded that genes controlling ovarian differentiations and functions are located on both arms of the X-chromosomes and the viability of the germ cells and oocytes is dependent on it. In addition, the occurence of familial 46,XX gonadal dysgenesis that is transmitted as an autosomal recessive trait suggests that at least one autosomal gene is essential for ovarian organogenesis e.g. development of rete-ovari or the synthesis or action of the putative meiosis-stimulating factor of the ovary (Buskov, 1978, Suppl.) could be under the control of an autosomal gene.

All the above explanation shows that XX complement in female and XY in male is necessary for normal sex development and differentiation. Any either numerical or structural anomalies of sex chromosomes affect the sexual development, differentiation and somatic features unrelated to sex.

Gonadal dysgenesis

Gonadal dysgenesis is a condition characterized by absence of germ cells from the gonads and the development of female genital ducts and external genitalia. Long before Turner described his syndrome in 1938. Several authors reported cases of congenital ovarian deficiency manifested by sexual infantilism and short stature in otherwise properly propotioned females (Neuhans, 1890; Olivet, 1923; Randerath, 1925; Baer, 1927; Schurmann, 1927; Rossle and Wallart, 1930; Bartalos et al, 1967). Later,

it became evident that this condition was due to primary ovarian deficiency rather secondary to pituitary deficiency by the demonstration of an elevated titre of urinary gonadotrophins (Varney et al, 1942; Albright et al, 1942).

The histological studies of Wilkins and Fleischmann (1944) revealed the presence of ovarian stroma with no follicles at all, hence the name ovarian agenesis. However, the discovery that most of these patients showed the absence of sex chromatin made the term gonadal agenesis seems to be more appropriate (Polani et al, 1954; Wilkins et al, 1954; Gordan et al, 1955).

The only constant clinical features of this condition are sexual infantilism, primary amenorrhoea and sterility. The fact that the short stature and other somatic anomalies are not invariably present allows us to divide gonadal dysgenesis into three clinical entities :

1. Pure gonadal dysgenesis, where there is normal stature and no somatic anomalies,
2. Gonadal dysgenesis with short stature as the only somatic anomaly.
3. Turner's syndrome, where short stature is accompanied by other somatic anomalies like webbed neck, shield-like chest etc.

Short stature is the commonest somatic abnormality associated with gonadal dysgenesis. Patients with short stature show a proportionate and generalized retardation of growth although the extremities tend to be slightly elongated giving an arm span greater than the height (Albright et al, 1942). Their birth weight tends to be low. In the majority of cases, retardation of growth is noted from birth and the patient may go on growing until 21 years of age (Gordan et al, 1955). The rest of the cases, however, may experience a sudden cessation of growth at the time of the appearance of scanty pubic and axillary hair (Albeux-Fernet and Deribreux, 1955). Some investigators believe that short stature is genetically determined (Graber, 1937; Varney et al, 1942). Others consider it to be a developmental defect like the dysgenetic gonads

(Wilkins and Fleischmann, 1944), which a few maintain that it is due to estrogen deficiency as estrogen treatment may promote further growth in these patients (Graber, 1937; Albright et al, 1942; Grumbach et al, 1955; Hausser et al, 1956).

Although patients may be of normal intelligence light to moderate intellectual impairment may be noted more in association with gonadal dysgenesis than in the population as a whole. Libido is generally reduced, but it can be restored to normal by the administration of estrogens.

At puberty, there is no menstruation or breast development and the patients are sterile. The axillary hair may be absent or very scanty; the amount of pubic hair is usually less than normal but more developed than the axillary hair.

The external genitalia are invariably infantile. The vulva has a rather flat surface. The labia majora are flat, while the labia minora remain thin and undeveloped. The clitoris is usually very small. However, in a few cases, virilism may be noted in the form of moderate clitoral hypertrophy and normal growth of pubic hair (Pich, 1937; Gordan et al, 1955; Grumbach et al, 1955). The source of androgen in these cases may be in the gonads (hilus cell hyperplasia), in the adrenal gland or in a aberrant adrenal tissue.

The external genitalia in this condition are normally developed but immature. The tubes are slender and the uterus is small and infantile, having either no endometrium at all or very scanty atrophic endometrium. The vagina is short and narrow with smooth thin walls; the vaginal smear fails to show estrogenic stimulation.

Gonads are represented by yellowish or white elongated streak-like structures on the posterior surface of the broad ligament running parallel to the tubes.

Microscopically, the steak is mostly formed of wavy fibrous tissue which is very similar to that of ovarian stroma. The streak is devoid of primitive germ cells or follicular apparatus and no structures resembling sex cords can be seen. The rete ovarii is invariably present and hilus cells are always seen in patients beyond the age of normal puberty (Jones et al, 1963).

A few cases described the streaks evidence of cortical proliferation with down-growths or nests of germinal epithelium and even occasional primordial follicles (Sellheim, 1924; Pela, 1935; Pich, 1937; Tronchi, 1938; Kerkhof and Stolte, 1956). This finding may account for the very occasional patient with slight breast development and the extremely rare case with a few menstrual bleedings.

Turner's Syndrome

In 1938, Turner described "a syndrome of infantalism, congenital webbed neck and cubitus valgus" in 7 females whose height ranged from 48.5 to 55 inches. The most frequently encountered anomalies are webbing of the neck due to folds of skin extending from the mastoid process to the acromion, cubitus valgus, shield-like chest with widely spaced nipples, coarctation of the aorta, multiple pigmented nevi, short fourth metacarpal bone and hypoplastic nails.

Subsequent studies of this syndrome and its variants have contributed to the understanding of sex differentiation (Simpson, 1976; Morishima et al, 1968; Grumbach, 1968; Engel and Forbes, 1965; Linsten, 1963; Mattovi et al, 1971; Palmer and Reichman, 1976; Hall et al, 1982).

The absence of a second sex chromosome (X-chromosome monosomy) is associated with four cardinal features :

1. female phenotypes,
2. short stature,
3. sexual infantalism owing to rudimentary gonads, and
4. a variety of somatic abnormalities.

Other somatic malformations associated with Turner's syndrome include webbing of the digits or of the axillae, senile facies, epicanthic folds, low set ears, bilateral cataract, depressed corners of the mouth, high arched palate, partial or total deafness, low hair line on the neck, tendency to keloid formation, renal anomalies idiopathic hypertension and intestinal telengiectasis.

This Turner's syndrome is given as a disorder occurring in the female with sexual infantalism due to streak gonads, short stature, lack of libido and at least two of the somatic anomalies mentioned above.

A few exceptional cases showing the clinical features of Turner's syndrome were reported to have normal or hypoplastic ovaries (Ulrich, 1930; 1949).

The gonadal dysgenesis was first used by Grumbach et al (1955) in reference to the rapid expansion of the phenotypic variation seen in rudimentary streak gonads. Hence, the Turner's syndrome has been widely identified with gonadal dysgenesis.

The first endocrinological investigations in these cases by Albright et al (1942) and Varney et al (1942) showed that the characteristic findings were an increased output of gonadotrophins. Such patients are phenotypically females but are (usually) chromatin negative. The discrepancy suggested a chromosome abnormality and this was confirmed by Ford and colleagues (1959), who demonstrated the 45,X karyotype.

The laboratory findings in gonadal dysgenesis

Estrogen levels are very low, vaginal epithelium show atrophy of the vaginal epithelium. The 17-ketosteroid urinary levels after puberty are usually low (3 to 6 mg per 24 hours), but not as low as those found in hypopituitary sexually infantile dwarf. In most cases, the levels of pituitary gonadotrophins are elevated; this elevation is noted only after the age of puberty. In a few cases, however, gonadotrophin levels may

be normal (Hauser et al, 1956; Greenblatt et al, 1956; Briggs and Kuperman, 1956; Elliott et al, 1959).

Only about 60% of the patients with Turner's syndrome have monosomy X. The remaining have a variety of karyotypes with a structural alteration of the X or mosaicism involving one or more cell lines with abnormal number or structure of the X-chromosome. The most frequent and commonly found of these is 46, X, i (Xq), that is an isochromosome for the long arm of the X. Deletions of part of Xp or Xq and ring X-chromosomes are not uncommon. The non-mosaics deletions of Xp are associated with short stature, whereas, deletions of Xq are unlikely to be associated with the short stature, but in general are associated with the presence of streak gonads and consequent infertility. Mosaicism accounts for about 40-50% of all the cases. About 10-15% are X/XX, or X/XXX and 10% or more are XX/X, i (Xq). These patients are sex chromatin positive in buccal smears, but most often fall in lower normal range and have a wide variety of clinical manifestation.

Jacobs (1979) reported about 1 per 10,000 phenotypic females. There is considerable loss of features with 45, X.

Jacobs (1979) observed 10% fall clinically recognisable spontaneous abortuses with 45, X constitution. As reported by the Carr (1971), the frequency of 45,X zygotes is 0.8%, probably the most common chromosome anomaly in humans, but less than 3% of 45,X chromosome conceptuses survive to term. Hook and Warburton (1983) reported a disparity in embryonic and fetal deaths between the 45,X genotype and those with mosaicism and/or an isochromosome for the long arm of X-chromosome (Xqi). They also observed that more than one dose of some locus (or loci) on the long arm of the X-chromosome may have a protective effect on the fetus.

Regarding the features of the Turner's syndrome, Donielle et al (1982) proposed "mapping" of the X-chromosome as follows :

The genes involved in gonadal function seem to be located on the proximal part of Xp and on the distal part of Xq; whereas, the genes whose absence is responsible for somatic features of the syndrome may be distributed along the length of Xp and the middle section of Xq (q21-q26).

Xq isochromosome patients are not always studied in detail as a result of their relatively frequent occurrence and phenotypic uniformity.

Xp isochromosomes, on the other hand, are often difficult to interpret, since the means of investigation and morphological studies utilized may not be precise enough to distinguish between a presumed i(Xp) and del (Xq).

Sex Chromosomes

Wyss et al (1982) concluded after reviewed karyotype and phenotype correlations that the hypothesis formulated about the X-chromosome by Sarto et al (1973), Therman et al (1974, 1976) and Fraccaro et al (1977), as well as those concerning phylogenetic evolution of sex chromosome morphology presented by Lyon (1974) and Hoo (1975), may receive support from almost all of the well documented, non-mosaic cases published to date. With regard to the gonadal and somatic features of the Turner's syndrome, the karyotype-phenotype correlations lead Wyss et al (1982) to propose "mapping" of the X-chromosome similar to Donielle et al (1982).

A practical supposition would be that, except in cases with minor del (X) (p 21) (p 22 - pter), only inactivation of a complete and unaltered X is compatible with completely normal gonadal function; this is evidently the case with the normal 46,XX woman, as well as in patients with balanced X/autosome and X/X translocations (monocentrics only). In this last category, normal gonadal function would also depend on a position effect as a consequence of the particular breakpoint on X. The presence of a normal second X is evidently essential for the

determination of normal gonadal function, probably both before and after inactivation.

Devidenkova et al (1978) observed amongst 209 patients with Shereshevesky-Turner syndrome, 69 women with structural aberrations of X-chromosome were detected 46,X,i(Xq) - 11, 45,X,/46, X, i (Xq)-24, 45,X/46,X, r(X)-14, 45, X/ 46 b (X or Y) - 10, 45,X/46, X, X, del (Xq) - 4; 45,X/46, X, del (Xp)-2; 45,X/46, X, idic (X)-2; 46,X, idic(X)-1, and 46,X,t(X,2)-1. All the patients with structural abnormalities of X chromosome were short in stature, but in no group was it as low on the average as in 45,X cases. Somatic signs were noticed in all structural changes of X, but they were less frequent and less pronounced. In some patients with r(X) and i(Xq), spontaneous menstrual bleeding and breast development were found.

The structural abnormal X chromosome appears to be functionally inactive, the phenotype of patients with structural rearrangements being close to the phenotype of patients with X-monosomy. At the same time, the abnormal X might have certain effects in early embryogenesis which mitigated the further development of the Shereshevsky-Turner syndrome. Parental age and birth order in the etiology of some sex chromosome aneuploidies were studied by Carothers et al (1978). The roles of maternal age, paternal age and birth order in the etiology of the 47, XXY; 47,XXX and 47, XYY aneuploidies were examined using the live born full sibs of proposite as controls. He, as in previous studies, also found the incidence of XXY's and XXX's was increased at high parental ages. A small but significant inverse relationship between parental age and the incidence of XYY's was also found.

Cytogenetic Findings : Turner's Syndrome (XO)

Polani and his associates (1956) postulated that patients with Turner's syndrome have only one X chromosome, since they have the same incidence of red-green colour blindness as much. This was verified later by Ford and his co-workers (1959); who published the first karyotype Turner's syndrome. They reported the presence of 45 chromosomes with

with one of the X-chromosome missing (Bartlos, 1970). The XO sex chromosome complement was rapidly confirmed by Stewart (1959), Fraccaro et al (1959), Tjio et al (1959) and Jacobs and Keay (1959). These patients are chromatin-negative.

In the following years, numerous XO patients were reported, showing that this chromosomal anomaly is the commonest one in gonadal dysgenesis. Ferguson-Smith (1965) was able to collect 117 patients with XO complements and concluded that short stature was common to all adult women with this chromosomal findings. The majority of these patients were found to have several of the somatic anomalies of Turner's syndrome, the commonest being shield-like chest, webbed neck, multiple pigmented nevi, short 4th metacarpal bones and hypoplasia of the nails.

Musculinization in the form of slight hypertrophy of the clitoris was noted in three cases (de la Chapelle, 1962; Forland et al, 1963; Ferguson-Smith et al, 1964).

Turner and Zanartee (1962) reported XO Turner's syndrome in two identical twin females.

A few patients with XO sex chromosome complements were shown to menstruate spontaneously, suggesting that some of these patients enter puberty with a few primordial follicles. This is elevated by the unusual case of Bahner et al (1960a,b) who was a chromatin-negative short phenotypic female having an XO complement, and yet she menstruated regularly and gave birth to a normal child.

Schwartz and Walter (1962) found an XO complement in a patient with Turner's syndrome and pseudohypoparathyroidism. She had shortening of the 4th metacarpal and metatarsal bones, exostosis of the auditory canal and failure to respond to parathormone.

Xiso-X

Correlation of karyotype with phenotype has become increasingly important as the numbers of cytogenetic syndromes has proliferated, in order to enable clinician to utilize available clinical clues as aids in differential diagnosis. This is exemplified in classical 45, X Turner's syndrome, which must be separated out from numerous other syndromes involving structural abnormalities of the X-chromosomes. One of the most confusing of these is the X-isochromosome-X syndrome, 46,X i(Xq) in which the patient presents with some or many of the features of 45,X Turner's syndrome. It has a positive buccal smear of the X-chromatin body.

An isochromosome for long arm of the X-chromosome was first described by Fraccaro et al (1960) in three female patients having primary amenorrhoea, underdeveloped secondary sexual characteristics and short but not webbed necks, all had low urinary levels of estrogen and elevated gonadotrophins. Their buccal smears were chromatin-positive and showed large sex chromatin bodies. Similar patients were described by Engel and Forbes (1961), Klotz and Xerace (1962), Hamerton et al (1962), Lindsten (1963), Sparkes and Motalsky (1963), Brown et al (1964) and Ferguson-Smith et al (1964).

Autoradiographic studies have shown that the isochromosome is the late replicating one (Muldal et al, 1963; Gianelli, 1963; Atkins and Santerson, 1964).

In addition to the 3 patients with the X-isochromosome X syndrome [46,X,i(Xq)] reported by Santana et al (1977). They reviewed the phenotype and cytogenetic finding in 41 previously reported cases. All 44 patients presented with short stature. Only 3 of the 44 had a webbed neck, when compared with 45,X patients, these 46,X i(Xq) cases had a lesser incidence of low posterior hair line, short neck hypoplastic nails, lymphoedema, congenital cardiovascular defects and high arched palate. There appears to be an unusual frequency of thyroiditis in the latter syndrome. The X-chromatin body was found in 30% or more cells examined

in all cases short structure appears as strikingly consistent feature both in the 46, X i(Xq) and the 45,X patients. The results in both 45,X and 46,X i(Xq) are similar with respect to short stature, streak gonads, lack of ovarian follicles, amenorrhoea, short 4th metacarpals and pigmented nevi. The frequency of mental retardation and partially developed nipples appears to be higher in the isochromosome cases. Other than these, the 46,X, i(Xq) patients exhibit for fewer phenotypic abnormalities than do the classical 45,X Turner cases. That such differences may exist is not surprising considering the profound cytogenetic differences between the 45,X and the 46,X i (Xq) karyotypes, former representing monosomy X and the latter representing trisomy for Xq and monosomy for Xp. An association between thyroid autoimmunity - and the X,i(Xq) syndrome has been suggested by several authors.

In the 14 patients with Turner's syndrome studied for growth hormone by Donaldson et al (1968) one had a 46,X i (Xq) karyotype. Carr et al (1963) reported histologically normal ovaries at postmortem examination in an 8 week old baby with a 46,X i (Xq) karyotype. That some patients enter puberty having enough primordial follicles and consequently enough potential for ovarian function may help explain ovulation and menstruation in classical 45,X Turner's syndrome, which must be separated out from numerous other syndromes involving structural abnormalities of the X chromosomes. One of the most confusing of this is the X-isochromosome X syndrome 46,X i(Xq) in which the patient presents with some or many of the features of 45,X Turner's syndrome, yet has a positive buccal smear for the X-chromatin body.

Some of these cases Nakashima and Robenson (1971) reported patients with 45,X Turner's syndrome who had normal menstruation, normal pregnancies and who had delivered normal babies. Due to the lack of normal follicles in streak gonads, it is expected that most 46,X i (Xq) patients will be sterile, but they should not be started on estrogen therapy until after an adequate opportunity for spontaneous menarche has been permitted. Above all observations permit one to make the general observation that

cytogenetics can be a potent tool in the differential diagnosis of primary amenorrhoea or infertility. The diagnostic stigmata which have recently been developed for this purpose make judicious use of chromosome studies (Khoo et al, 1972; Gelson et al, 1973).

$X\bar{X}$ (Short arm deletion)

This is a chromosomal anomaly where most of the short arm of one X chromosome is deleted. A patient with his karyotype was reported to have streak gonads, primary amenorrhoea, short stature, infantile external genitalia and no breast development (Jacobs et al, 1961).

de la Chapelle (1973) reported deletion of short arm of one X in the patient with short stature, public and axillary hair were normal, breast were short but feminine. The external genitalia were normal, vagina 7 cm. deep, small uterus (4 cm) and small ovaries were palpable.

$X\underline{x}$

In this chromosomal anomaly, deletion of the long arm of an X-chromosome exists (Jacobs et al, 1960; de Grouch et al, 1961 b; Becker et al, 1963 b). Although streak gonads are present in such patients, short stature and other stigmata of Turner's syndrome are not found. Jacobs (1969) suggested that short 4th metacarpal and pigmented nevi may occur in individuals with deletions of the long arm.

XX

The patients have been described with an apparently normal XX sex chromosome complement associated with evidence of gonadal dysgenesis (Jacobs et al, 1961; Aubest, 1962; de la Chapelle, 1963; Haddad, 1962, Lindsten, 1963; Jorro et al, 1963; Ferguson-Smith et al, 1964). Seven of these patients had short stature with some stigmata of Turner's syndrome. The patients with ovarian dysgenesis and a normal female karyotype (46, XX), there have been six reports of this syndrome being presented in more than one sibling in the family (David, 1980; Elliott,

1959; Josso et al, 1983; Hauser, 1963; Greenblatt et al, 1967; Christakos et al, 1969; Perez Ballester et al, 1970).

David Vasely et al (1980) reported details endocrinologic and genetic studies of a family of four sisters and one brother in which 3 sisters appear to have pure ovarian dysgenesis with amenorrhoea, lack of secondary sexual development and normal 46,XX chromosomal constitutions and who do not have any other congenital anomalies.

Two families with 46,XX chromosomal karyotypes have been reported in which one sister had ovarian hypoplasia and the second sister has had ovarian dysgenesis (Boozkowski, 1970). In addition to ovarian hypoplasia and Turner's syndrome in the differential diagnosis of pure gonadal dysgenesis one other entity should be considered. This entity is the so-called ovarian insensitivity syndrome in which estrogen levels are within or at upper end of normal range co-existing with tonically elevated LH (Jones, 1973). These patients are not in the menopausal age group and present with amenorrhoea and are characterized by having ovaries hyporesponsive to exogenous gonadotrophins. Previously described reports (Jarro et al, 1963; Christakos et al, 1967) of ovarian dysgenesis, 46,XX karyotypes and other congenital anomalies were of the product of proven consanguineous relationship. In one of the reports (Perez-Ballester et al, 1970) of deafness and ovarian dysgenesis with 46,XX chromosomal karyotypes, the patients heights were not recorded, while in other two reports (Josso et al, 1963 and Christakos et al, 1967) all sisters were under 152 cms in height. In one of the reports (Hauser, 1963) one sister had a congenital anomaly of a pelvic kidney and these two sisters were unusual in that their estradiol levels were not low as is usually seen in cases of ovarian dysgenesis but more like that of ovarian insensitivity syndrome.

Two of the three sisters with clinical presentations of pure gonadal dysgenesis reported (Vesely, 1980) to have streak gonads on laparotomy while the third sister has not had a laparotomy. A laparotomy should

probably be done in all patients with ovarian dysgenesis and a 46,XX chromosomal constitution, as the Y chromosomal component appears to make these patients a high risk (95%) for the development of germ cell tumour (Teter et al, 1951; Frasier et al, 1964; Marquez-Monter et al, 1972; Fischer et al, 1969; Josso et al, 1969). The patients with gonadal dysgenesis and normal female karyotypes or 45,X monosomy or with a mosaic pattern not containing a Y chromosome, on the other hand, run a low risk of developing malignant gonadal tumours (Marquez-Minter et al, 1972). Thus, the patients karyotype appears to be very important in determining which patients with gonadal dysgenesis need a laparotomy and surgical removal of the gonads and which do not. With regard to a laparotomy versus a laparoscopy in these patients, Sutton (1974) has pointed out that tissue removed by laparoscopic ovarian biopsies is not necessarily representative of the histologic appearance of the whole ovary and, thus, on these patients with 46,XY chromosomal constitution and ovarian dysgenesis, a laparotomy appears to be the procedure of choice.

Verilization has been found with a number of gonadoblastoma patients and verilization has been seen secondary to hilus cell adenomas in patients with gonadal dysgenesis and a 46,XX karyotype (Kaufman et al, 1966) as well as with 46,XY karyotype (de la Chapelle et al, 1962). Progressive verilization without germ cell tumours has been reported by Judd and colleagues (Judd, 1970) in a patient with 46,XX pure gonadal dysgenesis and the presence of hilar cells, and by Bardin and associates (1969) in a patient with 46,XY pure gonadal dysgenesis and the presence of leydig cell hyperplasia. Biochemical studies of gonadal venous blood indicated in both the cases that the source of androgen production was gonadal.

Familial ovarian dysgenesis in patients with a 46,XX karyotype (pure ovarian dysgenesis), there have also been reports of familial ovarian dysgenesis in sisters with 46,XY karyotypes (Brögger et al, 1965; Cohen et al, 1965; Sternberg et al, 1968; Stanesco et al, 1968; Espiner et al, 1970 and Fleishman et al, 1976) and one report of an ovarian dysgenesis in two sisters who apparently had mosaic karyotypes (Iazkovi et al,

1960). Thus, there appears to be more of a familial incidence of ovarian dysgenesis in those patients without Turner's syndrome as opposed to patients with Turner's syndrome, which is a disorder usually occurring sporadically (Engel et al, 1965; Goldberg et al, 1968). The case of Vesely (1980) of multiple affected sibs, along with case of multiple affected sibs of Elliott et al (1959), Greenblatt et al (1967), Perez-Ballester et al (1970), Christakos et al (1969) suggests that 46,XX gonadal dysgenesis is an autosomal recessive condition, as has been suggested previously by Christakos et al (1969). Thus, the data indicates that development of human oocytes and ovarian follicles requires genes on autosomes as well as genes on two X chromosomes. This autosomal recessive pattern for 46,XX pure gonadal dysgenesis differs from 46,XY pure gonadal dysgenesis where the familial pattern suggests X-linked recessive genes (Sternberg et al, 1968; Stanisco, et al, 1968).

XY

An XY sex chromosomal complement in a patient with pure gonadal dysgenesis was first described by Harnden and Stewart (1959). Their patient was a 19 year-old tall phenotypic female with primary amenorrhoea and increased urinary gonadotrophic excretion. The buccal smear was chromatin-negative and no drumsticks were found in the circulating leukocytes.

The same chromosomal finding was also reported by Netter et al (1960); de Grouchy et al (1960) and Stewart (1960). Wu et al (1976) reported patient with XY gonadal agenesis syndrome with absent gonads, uterus and fallopian tubes and a normal vaginal and slightly enlarged clitoris. XY gonadal agenesis is characterized by the absence of gonads and varying degrees of dysgenesis of the gonaducts and external genitalia. Overzier and Lindon (1955) reported these findings in a pair of siblings and used term "true agonadism" to describe these patients (Overzier et al, 1950). In two additional reports, the patients did not have complete evaluations to determine their genetic sex and the state of the gonaductal system; they may not be cases of "true agonadism"

The patients of these cases (Overzier et al, 1953; Schoen et al, 1955; Philipp et al, 1974; Chaptal et al, 1958; Sarto et al, 1973; Emson et al, 1965; Dewhurst et al, 1963; Rath et al, 1968; Parks et al, 1974) show very similar findings. They had absent gonads with no vagina, rudimentary or absent gonaducts, rudimentary and often fused labial/ serotal folds and a clitoris or larger phallic structure. Patient reported by Rios et al (1974) having normal external genitalia and a normal vagina; unidentifiable fibrotic tissue on the pelvic wall and inguinal ring. Patient lacks normal gonaductal structures and gonads. In nearly all forms of gonadal dysgenesis with or without Y-chromosome, there are usually recognizable gonaductal structure although they may be unilateral (Davidoff et al, 1973). In a reviewe of mixed gonadal dysgenesis, Davidoff and Federman (1973) reported that all patients with mixed gonadal dysgenesis and bilateral dysgenetic testis have a uterus, with the exception of a single case in which patient had no uterus, but had evidence of gonads in the form of a gonadocytoma.

Except in the case of congenital absence of the uterus regression of Mullerian structures probably occurs as a result of the action of Mullerian Regression Factor (MRF) produce by the fetal testis. The regression is usually complete by 8-10 weeks of life, at which time the external genitalia are still undifferentiated. Gonadal (testicular) failure at this time could result in female external genitalia and the absence of gonaductal structures. In the syndrome of XY gonadal agenesis, primary primordial gonadal failure is not sufficient to explain all of the findings. Since Mullerian regression has occurred, the testis or parts of the testis must have functioned normally for a brief period. Josso et al (1974) have demonstrated that it is possible to destroy primordial germ cells by irradiation of human fetal testicular tissue in vitro, without destroying MRF activity. A similar catastrophe perhaps resulting from exogenous toxins or viral infections, with subsequent atrophy of gonadal tissue, could theoretically be a cause of XY agonadism.

In the feminizing testis syndrome, there are Mullerian structures since these patients have functioning testis which still produce MRF. The

peripheral unresponsiveness to testosterone and the normal estrogen production account for the normal female external genitalia and secondary sexual characteristics one could separate that a patient with testicular feminization could develop the same findings as seen in the patient by Wu et al (1976) scanty of sexual hair, slightly enlarged clitoris, normal labia and no posterior fusion, normal vagina but no cervix, no gonads, no uterus or fallopian tubes were identified, the buccal smear was chromatin-negative; if testicular degeneration occurred prior to the onset of puberty Rios et al (1974) patient with XY gonadal agenesis was responsive to exogenous testosterone with an increase in nitrogen and phosphorus retention.

Parks and associates (1974) proposed that patients with XY karyotype, absent gonads and ambiguous external genitalia could be a form of dysgenetic male pseudohermaphroditism with varying amount of testicular tissue (Leydig cell function). Their 3½ year old patient had a significant increase of plasma testosterone in response to HCG stimulation, suggesting the presence of some Leydig cell remnants. In the different cases reported of "true agonadism" the five adolescents had elevated gonadotrophins in plasma or urine. In 2 cases, plasma testosterone levels were barely detectable of these two, one case of (Rios et al, 1974) had no response to HCG stimulation. While it is important to test for Leydig cell function; elevated gonadotrophin concentrations with low plasma T levels probably indicate that no residual Leydig cell function present.

Wu et al (1976) reported a 12th case of an XY genetic male with agonadism and the 2nd case with absent uterus, and fallopian tubes, associated with a normal vagina and almost normal external female genitalia. Twenty-four LH plasma pattern was quantitatively similar to that found in normal late pubertal subjects; but the values were in the castrate range. The testosterone and dihyrotestosterone were 14.4 ng/dl and 14.2 ng/dl respectively. Urinary estrogen excretion was 15 and 19 ug/day. These clinical and endocrine findings suggest that the patient was a genetic male in whom Mullerian regression occurred normally, however, the testis probably ceased functioning early in gestation preventing

normal development of the Wolffian system. This set of events resulted in agenesis of the gonaductal system and female external genitalia.

Xiso-x

An isochromosome for the short arm of the X-chromosome may be very similar to an X-chromosome whose long arm has been partially deleted. Such a small metacentric chromosome was found in patients (Lindsten, 1964; Polani, 1964). Phenotypically, they were similar to patients on the Xx group.

Mosaicism

XO/XX : A long list of case reports showing this type of mosaicism starts with that of de Grouchy et al (1961 a). A careful study of these patients shows that they may have a clinical picture ranging from that of a complete Turner's syndrome to that of normal female phenotype. It is presumed that the XX cell line has a diluting effect on the Turner's phenotype and that the proportion and distribution of XO cells are the deciding factors in the degree of variation (Ferguson-Smith, 1965).

XO/Xiso-X

The first patient with this type of mosaicism was reported by Blank et al (1961). Similar cases were reported by de la Chapelle (1962); Lindsten (1963); Gropp et al (1963), de Grouchy et al (1963); Court Brown et al (1964) and Ferguson-Smith et al (1964).

All these patients had short stature, but the incidence of somatic anomalies was lower than in the XO cases. One patient had clitoral hypertrophy associated with hyperplasia of hilus cells of the gonadal streak (Bergada et al, 1962).

XX/XY

This type of mosaicism was described in a chromatin-negative phenotypic female having streak gonads (Forteza et al, 1963).

XO/XXX and XO/XX/XXX.

Three patients have been reported with the double cell line and 5 with the triple cell line (Hayword and Cameron, 1961; Carr et al, 1962; Court Brown et al, 1964; Ferguson-Smith et al, 1964). Their phenotype was similar to that of the XO/XX group.

KLINEFELTER'S SYNDROME

Klinefelter, Reifenstein and Albright (1942) described a "syndrome characterized by gynaecomastia, aspermatogenesis without a Leydigism and increased excretion of follicle-stimulating homrone". Since that time, this disease entity has been the subject of several extensive studies culminating in the demonstration of a sex chromosomal aberration as the apparent etiological factor.

A distinction is usually made between "true" or chromatin-positive Klinefelter's syndrome and "false" or chromatin-negative Klinefelter's syndrome. It is felt, however, that what has been called false Klinefelter's syndrome is mostly some other form of male hypogonadism. If not stated otherwise, the term Klinefelter's syndrome in this monograph is used to refer to the various forms of the true variety. The following syndromes have been used to describe the syndrome (Overzier, 1963); true Klinefelter's syndrome (Nelson, 1956); testicular dysgenesis (Plunkett and Barr, 1956); true Klinefelter's syndrome (Nelson, 1956); female pseudohermaphroditism with gonadal dysgenesis (Nelson, 1956); chromatin-positive micro-orchidism (Ferguson-Smith, 1958); primary micro-orchidism (Ferguson-Smith, 1959); seminiferous tubule dysgenesis (Grumbach, Blanc and Engle, 1957); and medullary gonadal dysgenesis (Stewart, 1959).

Bishop and Polani (1960) formulated the criteria of Klinefelter's syndrome as follows : micro-orchida (descended, very small, usually firm testicles), gynaecomastia and markedly raised urinary gonadotrophin excretion. The findings in this syndrome were extensively studied by Hornstein (1963) who speaks of obligatory and optional findings. A

positive sex chromatin pattern and after puberty, bilateral testicular atrophy or hypoplasia are considered as obligatory findings. Increased gonadotrophin excretion and azoospermia or aspermia are regarded as "almost constant" findings after puberty. The degree of testicular hypoplasia and that of sperm production show wide variation. Gynaecomastia, which must be differentiated from lipomastia, is regarded as an optional findings (Hiller and Nelson, 1945). Overzier (1963) estimated its frequency to be 60%, while Hornstein (1963) found it in 10 of his 18 patients. The latter author noted side differences in the degree of gynaecomastia, in an additional case mental retardation is frequent finding in this syndrome.

Other findings in Klinefelter's syndrome include gouty arthritis and elevated uric acid level (Indemini and Ammann, 1961; Arduino and Glucksman, 1963) abnormally low I^{131} uptake frequently associated with poor response to TSH (Barr et al, 1960; Davis et al, 1963) and cerebellar atresia (Indemini and Ammann, 1961). Hoefnagel and Benirschke (1962) were impressed by the frequency of twinning in the sibships or families of patients with Klinefelter's syndrome, while Lubs (1962) noted a high frequency of malignancies among these patients.

This syndrome is generally manifested at adolescence where small firm testis with tubular hyalinization but normal number of Leydig cells, azoospermia, gynaecomastia, Eunuchoid habitus, elevated urinary gonadotrophins and low concentration of urinary 17 ketosteroids. Surveys of the prevalence of 47,XXY fetuses by analysis of karyotype in non-selected new born infants indicate an incidence of about 1/1000 males (Jacobs, 1979). No racial or geographic predilection has been observed (Hook and Hamerton, 1977). Only 0.1% of clinically recognizable spontaneous abortions have a 47,XXY karyotype (Jacob, 1979).

Meiotic non-disjunction seems to be the basis of the abnormal sex chromosomal findings in this syndrome. Increased maternal age and parental irradiation have been suggested as relevant factors in the etiology of this condition (Ferguson-Smith, 1963).

Depending on the somatic development, there are three types of patients with Klinefelter's syndrome (Hornstein, 1963).

1. Eunuchoid type, where long legs are most characteristic features
2. Dysplastic type, where the patients are of somewhat shorter stature
3. A type closely resembling the somatic norm.

External genitalia are of male type. The penis is normally formed and is often deminised in length but very seldom in diameter. A long prepuse is frequently seen. Erection and intercourse are usually normal in younger subjects. The scrotum is mostly smaller than normal and is frequently taut, poorly pigmented and has scanty hair. The pubic hair is of female type and reaches a little over the mons pubic and the vicinity of the root of the pubic. The internal sex organs are smaller than normal, with the testis of the adult measuring 1.0 to 2.6 cm in length (Raboch, 1957). The small epididymides, prostate and seminal vesicles, as well as the sparse pubic and facial hair, the somewhat high pitched voice and the precocious osteoporosis are considered, as the expression of a general androgen deficiency.

Zublin (1953) noted psychological peculiarities in Klinefelter's syndrome patients, these were later confirmed by several studies (de la Chapelle and Hortling, 1960; Dumermuth, 1961). It seems that mental retardation is a frequent finding in this condition and may be clue for the early diagnosis before puberty. With increasing age, one finds an increased expression of the "endocrine psychosyndrome" of Bleuler (1955), impulsive excitation and dejection states, deminished heterosexual interest, increased impotence and general loss of initiative. In Klinefelter's syndrome patients, according to Stumpfl (1960a,b) the relation to other sex is not a simple reaction to their own abnormal physical state but is primarily due to an alteration in the archetype of their "anima".

The microscopic changes affecting the testis are those of hyalinization, sclerosis and obliteration of most of the seminiferous tubules, focal

hyperplasia of mostly degenerated Leydig cells and a loss of the particular elastic fibers. The walls of the few persisting tubules are thinner and their lumina are filled with sertoli cells or undifferentiated cell elements. The hyalinization and sclerosis start to develop at puberty and are irregularly distributed, resulting in an irregular compression of the individual tubules. There is a marked deficiency or absence of germ cells. In exceptional cases, there is some evidence of spermatogenesis which can even result in fully developed sperms (Raboch and Bleha, 1960; Narman, 1961).

Between the hyalinized tubules there is a diffuse growth of Leydig cells, the majority of which show degenerative changes. Some cells may show coarse vacuolization or "hyaline droplets" in the cytoplasm and appear to be enlarged.

Testicular changes may also be recognised before puberty (Sibenmann and Prader, 1958; Ferguson-Smith, 1959). They consist mainly of a considerable difference in the diameter of the infantile tubes. With advancing age, there is a distinct diminution of the spermatogonia.

None of the hormonal findings are specific for Klinefelter's syndrome, although the increased gonadotrophin excretion in urine is a constant feature. The different estrogen fractions and the 17-ketosteroids are mostly normal or slightly decreased. There is a deficiency of androgens at all ages. After a long term administration of massive doses of chorionic gonadotrophin, the 17-ketosteroids and/or the estrogens may show a transitory increase (Leon et al, 1959; Seringe and Bach, 1961). This indicates that some functional activity of the Leydig cells is still present.

Polani et al (1958) studied the incidence of colour blindness among patients with Klinefelter's syndrome and suggested that these patients have two X-chromosomes.

Jacobs and Strong (1959) reported a 47,XXY - 24 year old chromatin-positive male with small testis, gynaecomastia, poor facial hair growth and high pitched voice. The same chromosomal findings were subsequently reported by several investigators (Ford et al, 1959a, b; Ford, 1960; Sandberg et al, 1960; Barr et al, 1960; Harnden, 1960; Lanmann et al, 1960; Ferguson-Smith and Johnston, 1960; Makino et al, 1960; 1963; Nowakowski et al, 1960; Court Brown et al, 1960; Leon et al, 1960; Bergman et al, 1960; Gray et al, 1961; Hustinx et al, 1961; Mamunes et al, 1961; Gropp et al, 1962; Rohde, 1962; Miller, 1962; Wright et al, 1963; Berg et al, 1963; Maclean et al, 1964; Ford et al, 1964; Jacobs, 1969; Hook et al, 1977).

Atkins and Gustavson (1964) showed the late replicating X to be smaller than its homologue in more than 50% of the cell examined.

The association of mongolism and Klinefelter's syndrome has been described in several cases. Ford et al (1959a) demonstrated 48 chromosomes with XXY and trisomy 21. Similar cases were also reported by Harnden et al (1960), Lanmann et al (1962); Maclean et al (1960); Gelderen and Hustix (1961); Hamerton et al (1962).

Mosaicism : XX/XXY

First reported by Ford et al (1959 b) and Nowakowski et al (1960). The patient's gonads were composed of XXY cell line with feminine or intersexual external or internal genitalia. Turpin et al (1962) observed a true hermaphrodite with ambiguous external genitalia with XXY/XX chromosomes. Crooke et al (1960) described XXXY/XX patient with small soft testis, no facial hair, scanty axillary hair, sparse and feminine type of pubic hair but no gynaecomastia.

Sandberg et al (1960); Sandberg (1961) reported XXY/XY chromatin-negative and Maclean et al (1961); Lubs (1962); Berg et al (1963); Wright et al (1963) and Warburg (1963) found chromatin-positive patients.

Fertility in Klinefelter's Syndrome

Spermatogenesis has been observed in scattered tubules (Bunge and Bradbury, 1956; Segal and Nelson, 1957; Jirasek and Raboch, 1958) and a few sperms have been demonstrated in the semen of patients with Klinefelter's syndrome (Ferguson-Smith, 1957). There is a case of a chromatin-positive male who had two sons (Warburg, 1963). Kaplan et al (1963) reported a 70 year old chromatin-positive XXY male with Klinefelter's syndrome having offspring. Lennox et al (1963) made reference to four chromatin-positive men with Klinefelter's syndrome who were supposed to have children. Subsequent analysis, however, invalidated this (Miller, 1964).

Incidence : General Population

Studying the frequency of Klinefelter's syndrome among patients attending infertility clinics, various authors have reported widely differing results. Among the possible explanations one is tempted to suppose that these results reflect the different composition of the groups who attend the different services. The percentage of Klinefelter patients among infertile males was found to be 8.5 % by Ferguson-Smith et al (1957). Close to this value is the finding of Jerasek and Raboch (1957) who reported 7 %. A much higher prevalence was noted by Nowakowski et al (1959) who found 20% of their patients to have Klinefelter's syndrome. Overzier (1961) reported a 10% incidence; while Jordan and Neermann (1962) found 2.4% of 1768 infertile male patients to have Klinefelter's syndrome.

Sex chromosome mosaicism

The words mosaic and chimaera have a long history of use of biology and sometimes have been employed synonymously. In human cytogenetics both connect with two or more chromosomally different kinds of cells. General definitions of the two terms are given by Anderson et al (1951). According to these authors a chimaera is "an organism whose cells are derived from two or more distinct zygote lineage". Whereas, a mosaic is formed of the cells of a single zygote lineage.

Since the first presumptive case of mosaicism involving the sex chromosomes was described by Ford et al (1959), it has become evident that mosaics are not only remarkedly frequent among subjects with abnormalities of the sex chromosomes but there is also a great variety of different types, involving either simple numerical difference or structurally altered X and Y chromosomes or both sex chromosomes mosaicism which leads to enormous phenotypic variation, depending on the proportion of the normal and abnormal cell types. Usually errors at anaphase like non-disjunction or lagging of the spindle are responsible for the origin of the variant cell lines in numerical mosaics.

In some new born infants assignments of sex is difficult or impossible because the genitalia are ambiguous, with those of the opposite sex. The anomalies vary through a spectrum from a mild form of hypospadias in the male to enlarged clitoris in the female with many intermediate states and many degrees of severity. Such problems do not necessarily involve abnormalities of the sex chromosomes, but may be due to single gene defects or environmental causes. However, determination of the karyotype is an essential part in the investigation of such patients.

Ambiguous genitalia or anomalies of the genitalia may also be seen in association with other dysmorphic features in a large number of malformation syndrome.

Hermaphroditism

Hermaphroditism in man implies a discrepancy between the morphology of the gonads and discrepancy of both gonads and genitals or ambiguous external genitalia. It is now well established that many chromosomal aberrations can result in ambiguity of the external genitalia.

In male fetus, once the indifferent gonad has differentiated into testis (5-6 weeks I.U.L.), it begins to produce hormones and musculinization of the fetus begins at about 8 weeks. During this period of musculinization (8 to 12 weeks) the fetal testis secretes 2 hormones. The first of these

is testosterone, as shown indirectly by correlation with cyto-differentiation of the Leydig cells, and directly by measurement of the testosterone concentration of fetal testis and plasma. Secretion of testosterone during this critical period of differentiation probably occurs in response to placental chorionic gonadotrophin. It appears that testosterone initiates virilization of the Wolffian duct into the epididymis, vas deferens and seminal vesicle. Testosterone is also converted by a 5- alpha -reductase to an active metabolite dihydrotestosterone, which causes virilization of the urgogenital sinus and the external genitalia. A functional androgen receptor controlled by an X-linked gene, is required for testosterone to give a masculine phenotype to XY individuals. When there is a defect in the synthesis of testosterone, normal musculinization may not occur, even when the testis has H.Y. antigen and there are normal androgen receptors.

The second hormone produced by the fetal testes is the anti-Mullerian hormone (AMH) - produced by the Sertoli cells. Though it has its effect only during a short critical period, it is produced from shortly after testicular differentiation until the perinatal period. AMH causes Mullerian duct to regress; in its absence they persist. It is clear therefore, that the female phenotype develops independently of the gonads. Normal female differentiation requires that there be no H-Y antigen, no testosterone, and no anti-Mullerian hormone; maleness is imposed upon a basically female potential by the hormones of the fetal testes.

True Hermaphroditism

A true hermaphroditism is an individual who has ovarian as well as testicular tissue. The presence of both types of gonadal tissue has to be proven histologically for the correct diagnosis of this intersexual state. The presence of viable sperm in the ejaculate, may be taken as a valid proof for the presence of testicular tissue. The two types of gonadal tissue may be separate, or they may lie in close proximity to each other when an ovotestis is obtained.

Depending on the distribution of the gonadal tissue, true hermaphrodites may be spoken of as :

1. Lateral hermaphrodites, where testicular tissue is found on one side and ovarian tissue on the other.
2. Bilateral hermaphrodites, where testicular and ovarian tissues (ovotestis) are found on both sides.
3. Unilateral hermaphrodites where testicular and ovarian tissue are found on one side and testis, ovary or gonadal streak is found on the other.

Clinical features

In most cases abnormalities of the external genitalia are noted at birth. However, these patients do not seek medical advice until puberty or prior to marriage. The external genitalia are usually ambiguous, showing a wide range of variation. As majority of cases have an enlarged phallus which usually hides the urethral opening. They are reared as males. The presence of a scrotum certainly adds support to this impression. The scrotum may be bifid or unilateral; it may be intact in the presence of a penile urethra. In about one-half of the cases an inguinal hernia containing a gonad may be found.

About 1/3rd of adult true hermaphrodites have females breasts, and about the same proportion menstruate (Overzier, 1963). The pubic hair distribution is usually that of virile women; a typical male distribution is less common. The growth of a beared depends on the hormone production. These patients are definitely sterile.

The testis or ovotestis may be any where along the normal route of descent of the testis. The most frequent location for the testis is in the scrotum. The ovary usually lies in its normal position or slightly lower.

The development of the Wolffian and Mullerian duct and external genitalia is governed by the nature and distribution of the gonadal tissue. The

embryonic testis in true hermaphrodites, as in normal individuals, is capable of suppressing Mullerian duct development on the corresponding side, while an ovotestis acts more like an ovary in this respect. As far as masculinization of the genitalia is concerned, both testis and ovotestis are competent, although the later has a weaker effect (Jones et al, 1965). This offers an explanation for rearing the majority of about 75% of true hermaphrodites as males.

The vagina varies in length and width. Its opening may be separate from that of the urethra or both may have a common opening. It may also open into a common urogenital sinus. The prostate is not always present (Overzier, 1955). The seminal vesicles, epididymis and other Wolffian structures show considerable variation in development.

The testicular component of an ovotestis is usually separated by a connective tissue membrane. Occasionally, a gradual transition is seen between one tissue and the other.

There is no hormone pattern typical of the true hermaphrodite. The 17-ketosteroid urinary secretion may be normal or decreased. In childhood this value is normal as the total of 17-ketosteroids is derived from the adrenal cortex without any contribution from the gonads. Following castration, this value is diminished. Pituitary gonadotrophins are normal or slightly elevated and their level rises after castration.

Cytogenetic findings

True hermaphrodites most of them are chromatin-positive. Hungerford et al (1959) were first to report chromosomal findings in true hermaphroditism, who found a 46,XX chromosome constitution in leukocytes. Sex chromatin studies on 12 different tissues of the body including the buccal mucosa were performed and found to be invariable in about 38% of the cells. One reported patient had gynaecomastia and a small penis which was within the normal size range and configuration for

a male in early adolescence. Surgery revealed the presence of an ovotestis in the right scrotal sac and testis in the left one. Harnden et al (1959); de Assis et al (1960); Ferguson-Smith et al (1960); Crossfield (1962); McGovern et al (1962); Dewhurst et al (1963); Overzier (1963); Solomon et al (1963); Bregaman et al (1963); Merril et al (1963); Jones et al (1965) found XX sex chromosome complement in true hermaphrodite. An XY sex chromosome complement was also reported by Grumbach et al (1960); Sandberg et al (1960); Domenix et al (1963).

Several mosaic patterns were described. Hirschhorn et al (1960) studied a patient with XY/XO mosaicism. Turpin et al (1962) found XX and XXY cell lines in a chromatin-positive infant with ambiguous external genitalia; an ovary, uterus and tube on the right side and scrotal testis on the left. Blank et al (1964) described a patient with a testis on one side and an ovary on the other with XX/XXYY mosaicism. Fraccaro et al (1962) observed triple cell line in an XX/XXY/XXYYY mosaic.

Waxman et al (1962) reported XX/XY mosaicism. The child showed posterior fusion of the labia minora, an enlarged clitoris and a short vagina.

Jones et al (1963) reviewed the pathologic and cytogenetic findings in 23 cases from the literature and added 6 of their own. Majority cases (24) were chromatin-positive and had 46,XX chromosome constitution.

Fitzgerald et al (1979) reported true hermaphrodite dispermic chimaera with 46,XX and 46,XY; karyotypes.

Willem et al (1981) reviewed 409 cases of true hermaphrodites. They found ovary on left side in 62.8% of the cases and testis on the right side of the body in 59.5% cases. The ovotestis was the most common gonad of the true hermaphrodite; amongst 806 gonads in 406 cases, it was found in 44.3%. They also reported that true hermaphrodites with 46,XX karyotype most commonly have an ovary on one side and ovotestis on

other side; these with Y-chromosome have a testis in 61% of cases. True hermaphrodites with 46,XX chromosome complement were chracterized by a male phenotype in 54% of cases, while 46% with a female phenotype.

Nithoul-Fekete et al (1984) observed ovary most frequently on the left side, an ovotestis and a testis more frequently on the right side.

Larry et al (1984) reported a 46,XX true hemaphrodite with a pure seminoma and H-Y antigen positive. Presence of H-Y antigen suggested them to believe the theory of autosomal translocation of sex specific genes or an undetected Y-bearing cell line (mosaicism).

MALE PSEUDOHERMAPHRODITISM

A male pseudohermaphrodite is an individual whose gonads are testes but in whom the differentiation of the external genitalia and genital ducts is deviated to varying degrees towards the female form.

Depending on the nature of the external genitalia and development of the secondary sexual characteristics, male pseudohermaphroditism are of two varieties :

I. **Masculinizing variety** : The external genitalia are ambiguous or predominantly masculine and the sex of rearing is either male or female. These patients show masculinization at puberty. They may have bilateral testis or a unilateral testis and a contralateral streak gonads. The latter entity is spoken of an asymmetrical gonadal differentiation (Bergada et al, 1962) or mixed gonadal dysgenesis (Sohval, 1963).

II. **Feminizing variety** : The external genitalia are feminine and the sex of rearing and secondary sex characteristics are female. A suitable term earned to this entity is testicular feminization (Morris, 1953).

Masculinizing variety of male pseudohermaphroditism

Clinical features

The presence of ambiguous external genitalia gives the first clue to the diagnosis of a hermaphroditic state. The sex of rearing is usually dependent on the severity of the condition and the judgement at birth. While these patients may be reared either as males or females, the latter seems to be the case in the majority of patients. At puberty masculinization is evidenced by enlargement of the phallus, deepening of the voice and hirsutism. The beard is present but stops growing earlier than usual. No breast development is noted and the physique is masculine. Patients with asymmetrical gonadal differentiation tend to be of short stature.

Male pseudohermaphrodites do not menstruate and are almost always sterile. Wulfsohn (1950) and Young (1951) have reported fertile cases.

Anatomical findings

Patients with bilateral testes : Bilateral testes are normal in size or small and they may lie in the labio-scrotal folds, inguinal canals or abdomen. Inguinal hernias containing the testes are not uncommon, and frequently the true sex of these individuals is revealed at herniorraphy.

In majority of cases, the Mullerian ducts do not develop. In some instances, however, these ducts may be well developed the presence of a uterus may give the first hint of the intersexual state. When a uterus is present, the testes occupy the position of the ovaries, they do not always have an epididymis, but if they do, a vas can be traced through the uterus to the vagina. Almost all cases have a rudimentary vagina which, in the absence of a uterus, is most probably of urogenital sinus origin. The external orifices of the urethra and vagina may be either common or separate. If a penile urethra is present, it is hypospadiac. A prostate surrounds the urethra and proximal part of the vagina.

Laboratory Data

Before puberty the 17-ketosteroid urinary excretion is just as high as in normal children. Following puberty the level may be nearly normal or low, depending upon the degree of testicular development. Estrogens are found in normal amounts for male. The pituitary gonadotrophins are generally slightly elevated.

Cytogenetic findings

Patients with bilateral tests : All of these patients are chromatin-negative and the majority have an XY sex chromosome complement (Lejeune et al, 1960; Alexander et al, 1961; Bergada et al, 1962; Cooper et al, 1963; Warkany et al, 1964; Jones et al, 1965). Among the mosaic pattern XO/XY seems to be the commonest findings (Ferrier et al, 1962; Klevil, 1962; Mellman et al, 1963; Robinson et al, 1964; Jones et al, 1965). Miles et al (1962) reported a case with two cell lines, one showing an XO complement and the other X and Y chromosomes together with a deleted X-chromosome, XO/XY mosaicism, where a deleted Y was noted in one of the cell lines, has also been reported (Jones et al, 1965). It is to note in these mosaics that they showed uterine development which suggests that the embryonic testes developing in the absence of a Y-chromosome from one of the cell line is not competent enough in suppressing Mullerian duct development.

Shah et al (1961) described a mentally retarded chromatin-positive male pseudohermaphrodite whose chromosomal complement in skin was 46-XX. This patient was included under the heading XX-males.

Patients with asymmetrical gonadal differentiation

In these patients, XO/XY mosaicism seems to be the commonest chromosomal findings (Netter et al, 1962; Willemse et al, 1962; Bergada et al, 1962; Job et al, 1963; Conen et al, 1963; Greenblatt et al, 1964; Zourlas et al, 1965 b). Warkany et al (1963) found XO/XXXY mosaicism in a child with low set ears increased carrying angle, infantile testes in inguinal region, right side presence of fallopian tube and uterine fundus but no gonad.

Schuster et al (1962) found three cell line (XO/XX/XY) with a streak gonad on the left and a rudimentary testis on the right. Klevit et al (1963) reported a patient with 3 cell lines having 45, 46 and 47 chromosomes. It is evident that an XO cell line is common to all the mosaic patterns of patients with asymmetrical gonadal differentiation.

Feminizing variety of male pseudohermaphroditism (Testicular feminization)

Testicular feminization is a hereditary condition which affects genetic males having testes and yet are phenotypic females with excellent breast development. There is a strong familial predisposition to this syndrome, and patients usually have sisters or aunts whose histories are suggestive of this intersexual state. Transmission through maternal line and carriers are usually normal females but decreased axillary or pubic hair and sometime delayed menarche (Puck et al, 1960) may be noted in apparently normal mothers, sisters, aunts or grand-mothers. This condition could be determined by either X-linked recessive gene or a male-limited autosomal dominant one (Jacob et al, 1959; Morris et al, 1963; McKusick, 1964). These possibilities may be difficult to distinguish since the affected individuals are sterile.

Goldberg et al (1948) were the first to recognise the typical picture. Morris (1953) coined the term testicular feminization and outlined the clinical picture clearly.

Clinical picture

Patients with testicular feminization phenotypic females who have excellent breast development which begins at the time of normal puberty. Menstruation does not occur, and in the majority of cases pubic and axillary hair growth is absent or scanty. The voice is quite feminine. Amenorrhoea ,sterility ,hernia or a tender mass in the inguinal region may lead lead the patient to seek medical advice. The intelligence of these patients is usually above average. The balance in a normal feminine manner and can had the normal sexual life of a woman and experience

orgasm (Morris, 1953; Walkins, 1957; Hauser et al, 1957). Following castration, libido is diminished (Witschi et al, 1942; Wilkins, 1957).

Physical examination shows that these patients are quite attractive females who are usually tall. The limbs, hands and fingers are usually long. The breasts are very well developed, but the nipples are small and poorly pigmented. Axillary and pubic hair is absent or scanty in the majority of cases. The scalp hair is normal or thick. No hersuitism or balding is noted.

The internal genitalia are quite feminine with a normal or small clitoris. The labia may be underdeveloped, especially the labia minora, and the labial hair is absent or scanty. In number of cases, the clitoris has been somewhat enlarged and the secondary sexual characteristics have not been well developed. This entity has been referred to as "incomplete" or "partial" testicular feminization (Prader, 1957; Nunez et al, 1960; Morris et al, 1963; Philip et al, 1965).

Vagina ends blindly and is of varying depth. Most cases are satisfactory for marital relations, but a few require vaginoplasty to lengthen the vagina. The internal genitalia are usually absent except for rudimentary uterine and other anlagen (including sometimes fallopian tubes and spermatic ducts) and for gonads which may be intra-abdominal or may be along the course of the inguinal canal and in the labia majora. More than one-half of the reported cases had hernias or inguinal gonads.

The first symptom which causes adult patients to consult a physician is primary amenorrhoea. Children are brought with the presence of unilateral or bilateral inguinal masses that turn out to be testis. Plasma levels of testosterone and urinary excretion of 17-ketosteroids are normal.

The defect in testicular feminization is end organ unresponsiveness to androgens due to complete absence of androgen receptors in the cytosol of

the appropriate target cells. The receptor protein is specified by the normal allele at the locus has called TFM a major sex determining gene of man "the master regulatory gene of the extragonadal sex determining mechanism".

Incomplete testicular feminization

A common variant of this syndrome is one in which the affected individuals show axillary and female pubic hair growth, underdeveloped breasts, a masculine change of voice and virilization at puberty. There is incomplete form of androgen sensitivity. In all other respects, they resemble the typical form of the disease. This variant is known as the incomplete form of testicular feminization.

17-ketosteroid urinary excretion is normal or elevated. Urinary estrogens show normal male or female values and cornification is noted in the vaginal mucosa. Pituitary gonadotrophins are normal or slightly elevated following castration the 17-ketosteroid and estrogen levels fall, vaginal cornification diminishes, and gonadotrophin levels rise.

The level of testosterone, dehydroepiandrosterone sulphate and androsterone sulphate in the peripheral blood are within normal male values (Pion et al, 1965).

Cytogenetic findings

As a rule, patients with testicular feminization are chromatin-negative and have 46,XY chromosome constitution (Jacobs et al, 1950; Stewart, 1959; Puck et al, 1960; Lejeune et al, 1960; Chu et al, 1960; Alexander et al, 1961; Barno, 1962; Morris et al, 1963; Zourlas et al, 1965a; Pion et al, 1965; Philip et al, 1965). Miller (1964) reported a mosaic with XO / XY / XX cell lines. While Forsberg et al (1965) reported a chromatin-positive (18%) patient who was mentally retarded and showed XY/XYY/XXY mosaicism.

Female Pseudohermaphroditism

Congenital adrenal hyperplasia

This is a condition characterized by ambiguous external genitalia in new born female infants and masculinization, rapid growth in height, marked muscular development and accelerated epiphyseal ossification in children. It may manifest itself clinically in one of three forms : (1) simple virilism, (2) virilism and sodium loss and (3) virilism hypertension.

The adrenogenital syndrome is a disturbance in which the genitalia and the whole physique is masculinized by an increased secretion of androgens from the adrenal glands. This is an inborn errors of metabolism due to an autosomal recessive mutant gene. Both sexes may be affected, but in females, the condition is more readily recognised at birth due to the concomitant pseudohermaphroditic state. In the usual form of the disease, a deficiency of the enzyme 21 hydroxylase causes a block in the synthesis of cortisol by the adrenals. This leads to an accumulation of excessive amounts of androgenic precursors and their metabolite and formation of excessive amounts of androgenic steroids. In such cases salt losing crisis may be very severe. Affected baby girls frequently have major anomalies of the external genitalia often to the point that an assignment may be impossible. The clitoris may be enlarged and the labia majora rugose and even fused. In males the same genotype produce premature virilization but there is no difficulty in identifying sex.

The external genitalia of a female fetus may also be masculinized if the fetal circulation contains excessive amounts of either male or female sex hormones. These hormones reach the fetal circulation from the maternal circulation and may originate either endogenously or exogenously. Androgenic hormones may be present in excessive amounts if the mother's adrenal cortex is over active or if she has received hormone therapy for example, some progestins used to prevent spontaneous abortion.

Laboratory data

17-ketosteroid urinary excretion is consistently elevated. Estrogens are

found in larger quantities than normal (Migeon et al, 1952); but the estrogen target organs are atrophied. These elevated values return to normal after treatment with cortisone. Migeon (1953) has presented information indicating that FSH is absent prior to therapy but appears after cortisone treatment.

Agonadism (Embryonic testicular regression syndrome / vanishing testes syndrome)

A variety of terms have been used to describe the spectrum of genital anomalies resulting from cessation of testicular function during the critical stages of male sexual differentiation i.e. 8 to 14 weeks gestation. The term vanishing testes syndrome is used to describe this heterogenous group of male pseudohermaphroditism because the findings suggest that testes vanish (for an obscure reason) before the completion of male sexual differentiation. These patients have a 46,XY karyotype. Gonadal elements are absent and the differentiation of the genital ducts, urogenital sinus, and external genitalia is variable. At one end of the spectrum is the group of patients with female external and internal genitalia in whom the deficiency of embryonic testicular function presumably occurred before 8 weeks of gestation (Cleary et al, 1977). Either lack of or deficient function of the fetal testis between 8 and 10 weeks of gestation would lead to ambiguous genitalia and variable development of the genital duct, from complete absence of both Mullerian and Wolffian ducts to partial development of either. This form of dysgenetic male pseudohermaphroditism has been referred to by some as the 46,XY gonadal agenesis syndrome (Sarto and Opitz, 1963; Edman et al, 1977; Coulam, 1979). Loss of testicular function after the critical phase of male differentiation (about 13 to 14 weeks) results in anorchia - a syndrome characterized by normal male differentiation both internally and externally but no gonadal tissue.

Abortions and Chromosomes

The problem of pregnancy wastage presents a legitimate field of medical inquiry which has attracted the interest of physicians and other

investigators for a considerable time. The fact that "defective germ cell plasma" as well as environmental factors lead to abortions was convincingly demonstrated by Mall and Mayer (1921). As the result of an extensive long term investigation, Hertig and co-workers (1959) attempted to calculate the percentage loss of the products of fertilization in man at different stages of their development. They found that the greatest ovular loss was in the pre-implantation stage; the next greatest loss was during the week following implantation, while the ovular loss after the first missed period was probably as great as 28.6% which is roughly comparable to the clinical abortion rate.

The cytogenetic techniques have been utilized in the study of spontaneous abortions. Such studies were performed on fetuses belonging to the category of "28.6% ovular loss" of Hertig et al (1959). The fact that chromosomal aberrations might be a possible cause of repeated abortions was suspected in the sibships of individuals with various chromosomal anomalies. Schmid (1962) and Carr (1963 a) were among the first to demonstrate that chromosomal translocation can contribute to repeated abortions, while Beshan et al (1964) reported chromosomal mosaicism (XO/XX) in a mother as the possible cause of such abortions. These reports, as well as the findings of complete triploidy in 2 still-born infants (Penrose and Delhanty, 1961; Delhanty et al, 1961) gave an impetus to systematic cytogenetic studies on spontaneously aborted conceptuses.

The incidence of chromosomal abnormalities varied from 22% (Carr, 1965) to 64% (Szulman, 1965). The study of cases of spontaneous abortion by various investigators showed that 27.9% of them had various forms of chromosomal anomalies (Clendenin and Bemischke, 1963; Thiede and Salm, 1964; Hall and Kallen, 1964; Szulman, 1965; Carr, 1965). This is certainly over 60 times the incidence of chromosomal anomalies at birth.

The maternal age in these cases is not different from that of women producing abortuses with normal chromosomes. The only exception, however, is that mothers who have trisomic abortuses tend to be slightly

older, which is in accord with the observation that trisomy in new born infants becomes increasingly common with increasing maternal age (Carr, 1965).

The average length of gestation for the chromosomally abnormal cases is usually shorter than that of chromosomally normal abortions. This is supported by the fact that only a few cases have been encountered with chromosomal anomalies in pregnancy (Aula and Hjelt, 1962; El Alfi et al, 1964; Carr, 1965).

The Geneva Conference (1966) concluded that chromosomal abnormalities were a significant factor in spontaneous abortions, since 19% of nearly 800 abortuses were found to have a chromosome anomaly. Almost all autosomal anomalies either numerical or structural were reported as a significant factor in spontaneous abortions.

Anomalies of the sex chromosomes

Carr (1965) observed monosomy of X chromosome. Szulman (1965); Clendenin et al (1963); Thiede et al (1964); Boue (1974); Arakaki et al (1970) reported monosomy in spontaneous abortions. Monosomy X (45,X) represents one of the most frequently encountered anomalies in spontaneous abortions, accounting for 15-25% of all anomalies (Warburton et al, 1964). Monosomy X is observed at birth, where it is expressed as Turner's syndrome, and is the least frequent of the sex chromosome abnormalities; 0.4 for 1000 new borns in contrast to trisomy X (47,XXX) 12 per 1000; Klinefelter's syndrome (47,XXY), 2.1 per 1000; and 47,XYY, 1-3 per 1000. This difference at both between the low frequency of monosomy X and the higher frequency of anomalies with extra sex chromosome can be explained by the fact that the majority of monosomes X are lethal. Furthermore, a large proportion of living subjects with Turner's syndrome are mosaics, whereas, in abortions massives are rare. From these results, it can be estimated that less than 5% of conceptions with monosomy X will go to term.

Haematological Malignancies

Application of cytogenetic study on these lines proved to be a useful tool in understanding the pathogenesis of previously obscure conditions, specially congenital abnormalities, mental retardation and sterility.

An important offshoot of these clinical applications was discovered by a keen observation by Hungerford and Nowell (1960) of a consistent group chromosomal abnormality later named Philadelphia (Ph^1) chromosome, in the patients of chronic myeloid leukemia (CML). This prompted an intensive search for other non-random chromosomal abnormalities in all other haematological malignancies. The initial enthusiasm began to wane when no additional consistent observations were detected. This quest for consistent cytogenetic abnormality in haematological malignancies got a big boost with the introduction of the banding techniques. This improvisation in the staining methodology helped in exact detection and localization of the chromosomal aberrations. This followed a resurgence of interest in the cytogenetic study.

In last couple of decades, many non-random cytotenetic aberrations are reported in haematological malignancies. The clinicians in collaboration with the cytogeneticists have started understanding the diagnostic, prognostic and therapeutic importance of chromosomal aberrations in haematological malignancies. Other important aspect, under the close study, is the possible correlation of chromosomal changes and oncogenesis. With the expanding knowledge in this field many new facts are likely to emerge which might help in understanding the subject of oncogenesis - in general and haematological malignancies in particular.

In 1960, two groups of investigators from both the sides of Atlantic produced evidence of a small G chromosome in the bone marrow cells of the patients suffering from chronic myeloid leukemia (CML). This chromosome was named Philadelphia chromosome (Ph^1) after the city where it was first reported by Hungerford and Nowell (1960). The other investigator to report this, was Baikie (1960). The discovery of the Ph^1

chromosome at a consistent abnormality in CML aroused sudden interest and optimism about a possibility of correlation between the causation of human cancer and chromosomal anomalies. The main interest was focussed on the possible significance in management and prognosis. This led many investigators to perform series of chromosomal studies on the bone marrow cells of the patients with leukemia, hoping to find a direct correlation between the occurrence of abnormalities and cause of other diseases. When it became apparent that chromosome changes in other diseases; especially haematological malignancies were neither specific nor non-random, the initial enthusiasm began to flag. This account of prebanding era. At this time, even the number of Ph^1 chromosome or the fate of the deleted arm was not known.

With the introduction of banding technique there was resurgence of interest in the cytogenetic studies. Casperson et al (1970) identified the partially deleted G chromosome in CML as a 22 not a 21 as had earlier been assumed. Rowby (1973) showed that the long arm of chromosome 22 had not benn lost from the cell but has been translocated on to the long arms of chromosome 9 and this is now recognized as the standard translocation t (9:22) (Q34, Q11) which form Ph^1 chromosome.

Individual chromosomal identification is also providing information about certain non-random abnormalities in chromosomes in acute leukemia and lymphomas.

In the field of cytogenetics and oncology the main area of study is restricted to haematology; principally the myeloproliferative and lymphoproliferative disorders.

In the haematological malignancies, patients with chronic myeloid leukemia (CML) have been the key stone for karyotypic analysis of human cancers. With the advancements in the technique acute leukemia and lymphomas are also getting considerable attention.

Chronic Myeloid Leukemia (CML)

The observations of a consistent small G group chromosome, later named Philadelphia (Ph^1) chromosome, by Hungerford and Nowell (1960) triggered one of the most intensive study in cytogenetics. But till 1973, the question whether the deleted portion of the long arm was missing from the cell or whether it was translocated to another chromosome could not be answered, mainly because it was impossible to identify each human chromosome precisely. Furthermore, the identity of this chromosome as either No.21 or No.22 could not be established. Still Ph^1 chromosome remained one of the very useful marker in CML and its association with better prognosis was well understood.

Casperson et al (1970) and O'Riordam et al (1970) confirmed the Ph^1 chromosome as No.22q-chromosome. Observation by Rowley (1973) Ph^1 positive CML patients and studied with Q and G banding, of presence of addition of small segment on chromosome No.9 (Rowley, 1973) raised the possibility of (9:22) translocation. Further, studies have led to reporting of many interesting and non-random aberrations with different findings in both chronic and blastic phase of CML.

Chronic phase of CML

Bone marrow cells from approximately 85% of patients of a typical chronic phase (CP) of CML show the Ph^1 chromosome (Whang Peng et al, 1968). The other 15% have usually normal karyotype i.e. Ph^1 negative, although abnormalities such as an extra C-group chromosome have been observed (Cancellos, 1976). These chromosomal changes line Ph^1 positive cell line in CML is observed in all myeloid cell series. But the chromosome obtained from PHA stimulated lymphocytes of patients with Ph^1 positive CML are normal (Lawler, 1977).

After the origin of Ph^1 chromosome as 22q - was settled (Casperson, 1970) and the report that the deleted piece is translocated to chromosome 9 (Rowley, 1973) the investigators were focussed to chromosome 9. From staining characters, it was proposed that the amount deleted from 22 is

transferred to 9. Same is confirmed by DNA content of both chromosomes : 9 and 22 by Sandberg (1980). In 85-90% of the patients with leukemia in whom this anomaly is found, it is due to a translocation involving chromosomes 9 and 22. In about 5% (probably less) of the cases of Ph^1 positive CML is due to a translocation involving chromosome or chromosomes other than 9.

In the first International Workshop on Chromosomes in Leukemia in 1978, 8% of the totally unselected cases showed variant translocations. Mitelman (1981) reviewed 107 cases. In this study 42 had simple (unusual) translocations and 65 had complex translocations.

Chromosome abnormalities in addition to the Ph^1 translocation are found in upto 3% of the patients with CML (Hayata et al, 1971; Mitelman et al, 1978).

In acute phase (blast crisis) of CML : When patients with CML enter the terminal phase, additional chromosomal abnormalities are superimposed on the existing Ph^1+ cell line in 90% of the patients. In a number of patients (20%) the 46, Ph^1+ cell line remains unchanged. The change in the karyotype preceded the clinical signs of blast crisis by 2 to 4 months. In general, if patients have a clone of Ph^1+ cell with a unique marker during chronic phase, this clone will be the one involved in the transformation.

Rowley (1980 a) studied 242 patients with Ph^1 positive CML by banding technique; 40 showed no change in their karyotype whereas, 202 had additional chromosomal abnormalities. He found 47 to 52 numbers of chromosomes. He reported chromosome No.8, 17, 19 and 22 most commonly involved. Mitelman and Levan (1981) reported in their study on 361, No.8, 17 and 22 were found to be the most commonly involved chromosomes.

Abnormalities of the sex chromosomes in the acute phase occur relatively rarely. Hossfeld (1973), Lawler (1977), Brandt and Mitelman (1977)

studied the site of origin of these new aberrant clones. Brandt and Mitelman (1977) reported the chromosomal changes of patients going into acute phase first appeared in the spleen. There was higher proportion of abnormal clone in splenic tissue than bone marrow (Hossfeld et al, 1973).

Chronic Lymphocytic Leukemia (CLL)

CLL is the most common type of leukemia in the western world but it is rare in the eastern region. It characteristically occurs in older patients with male predominance of 2:1. This CLL is characterized by abnormally high numbers of lymphocytes in absence of any chronic or viral infection. 90% of the cases show affection of B-lymphocytes, remaining 10% have either T cell CLL or other variant. Predominancy of B-cells resulted into the change in immunological status.

The cytogenetic study of CLL is greatly complicated by the low mitotic activity of the bone marrow and poor response to in-vitro stimulations. Also, since CLL is thought to be a primary B-cell neoplasm, the findings of the in-vitro peripheral blood studies using PHA (phytohaemagglutinin) or Pokewood mitogen (which are mainly T cell stimulators) are difficult to interpret. Due to same reason almost all cases reported have exhibited normal diploid karyotype. More recently, a variety of mitogens have been used with an emphasis on those that stimulate B-cells, such as lipopolysaccharides and Epstein Barr (EB) virus (Autio et al, 1979 and Gahrton, 1980). These more recent studies have revealed a variety of chromosomal abnormalities.

Gunz et al (1962) reported the observation of the Christ Church (Ch^1) chromosome (Gp-) in several members of the family of which two had CLL. Originally it was thought to bear the same relationship to CLL as the Ph^1 did to CML. Subsequent studies showed no Ch^1 chromosome in any other case.

The studies of 30 cases showed chromosomes 12, 14, 17 were involved in aberrations in 23.3% and 26.7% of the cases, all these being above one standard deviation (20.9%).

Nine cases out of 11 cases with involvement of chromosome 14, displayed a mar 14q+, an abnormality in which material from some other chromosome is translocated to the end of No.14 producing this abnormality which is similar to t (8 : 14) described for lymphomas. These 9 cases include translocations between 14 and 8 (2 cases), 11 (2 cases), 12 (one case), 14 (3 cases) and 18 (one case). Engagement of 17 was noted in 8 cases, including one case with an isochromosome 17q (Nowell et al, 1978).

Acute Leukemia

Chromosomal abnormality was first reported in acute leukemia by Ford et al (1958). Though, immature cells were easily available, technical problems hampered these studies, specially the variable mitotic yield to fuzzy chromosome. It also became apparent that approximately 50% of patients had normal karyotype and the rest which showed chromosomal abnormalities were all random in nature. Hence, the chromosomal abnormalities in acute leukamia were considered as an epiphenomenon.

Acute Myeloid Leukemia (AML)

This group includes all the types of acute non-lymphocytic leukemia (ANLL). The cytogenetic changes in ANLL were extensively reviewed at the first (1977) and the second (1979). International Workshop on Chromosomes in Leukemia.

Mitelman and Lewan (1981) presented a collection of 497 cases with chromosomal aberrations. The chromosomes most commonly involved in those aberrations either alone or in combination were No.5,7,8,19 and 21. Incidence of aberration of No.5 (17.1%). At least one of these five chromosomes is involved in aberration in 389 of total 496 cases (77.4%). When the type of chromosome change is taken into account, non-random was of the abnormalities becomes even more obvious.

From the study, it is evident that aberrations of the same chromosome are of similar kinds. Thus chromosome 5 is affected as deletion of the

long arm in 47% and as monosomy in 31%, whereas, chromosome 7 is affected as long arm deletion in 22% and as monosomy 65%. In contrast loss of genetic material from the chromosome 8 is rare and it accounts for only 8% of the cases, whereas, it becomes trisomic in 57% and is engaged in translocations in 35%. Chromosome 17 is engaged in translocation in 55% and becomes trisomic in 14% including 8 patients with 17q. This chromosome is lost in 30% of cases. Chromosome 21 is apparently trisomic and monosomic equally often, but it is observed that this chromosome is involved in various translocations in about 50% of the cases. Thus, an overall gain of chromosome 8 and the 20 of 7 are the most frequent numerical chromosomal changes.

8, 21 translocation was first reported by Kamuda et al (1962) in 1968. Rowley (1973 a) reported a balanced translocation between Numbers 7 and 21. The 8, 21 translocation is frequently associated with the loss of a sex chromosome (Sakuri and Sandberg, 1976 a). 32% of the males with t (8, 21) are -Y and 36% of females are -X (Second Workshop, 1980). (15; 17) (q25, q22) was first reported in acute promyelocytic leukemia (M_3 type AML) by Rowley et al (1977). The peculiarity of this translocation is its presence only in M_3 type of AML and is not seen in any other type of acute leukemias, hence, it has a diagnostic significance.

Acute Lymphocytic Leukemia (ALL)

ALL is mainly a disease of childhood. The diagnosis of this condition is based on morphological basis of stained bone marrow smear. The morphological FAB classification divides into L1 - childhood type, L2 heterogenous group and L3 rare hemogenous group with cytomorphology of Burkitt's lymphomas. It is now well established that in ALL, as in AML about 50% of the patients have clonal chromosomal abnormalities. The remaining patients have normal diploid karyotypes (Oshimura et al, 1977a; Cemino et al, 1979 and Sandberg, 1980). The chromosome types most commonly affected were numbers 1,6,8,9,14,21 and 22. No.22 and 14 were found to be involved most frequently.

Hodgkin's Lymphoma

Hodgkin's lymphoma was the first lymphoma in which chromosomal abnormalities were demonstrated (Springs et al, 1962). There are also number of studies in pre-banding era (Lawler, 1973 and Atkin, 1973). However, detailed chromosome studies using banding techniques are scanty. This is mainly due to the difficulty in getting good banding of the neoplastic cells, complexity of the changes in these cells are scarcity of such cells in metaphase. The prominent finding is a hyperploid state in Hodgkin's lymphoma. Sandberg and Hossfeld (1979) found that 38 cases had modes in the triploid and tetraploid range as compared with 10 cases with near diploid modes. In confirmity with the probable B cell origin of the neoplastic cells 14q+ chromosomes have been found quite frequently in HL (Reeves, 1973; Fukuhara et al, 1976 and Fukuhara and Rowley, 1978). The chromosome studies show that the abnormal cells are clonal in origin and many patients have mosaic population with near diploid and triploid or tetraploid cells. It is also noted that the percentage of normal metaphases tends to be lower in patients with lymphocyte depletion than in those with mixed cellularity type HL. It is also seen that the frequency of normal metaphase is still higher in patients with lymphocytic predominent HL. Based on this observation, it is noted that the higher number of normal metaphase carries better prognosis.

Non-Hodgkin's Lymphoma (NHL)

Cytogenetic study by Mark Ekedanl and Dahlenfor (1978) of 45 cases of their own and from literature show some generalization. The chromosomes most frequently involved are numbers 1,3,7,8,11 and 14.

Among the numerical changes, there was frequent gains of chromosomes 3 and 7 and losses of chromosomes 6,8 and 15; while the most frequently involved chromosomes in structural changes were 1,3,11 and 14 (Reeves, 1973).

In NHL chromosome 14q+ is also reported quite frequently.

Multiple Myeloma (MM)

Multiple myeloma is affecting plasma cells and leads to the production of monoclonal proteins. Bone marrow cells of these patients have appeared to have a normal karyotype. This may be related with very low mitotic index of the myeloma cells, so that the only cells in division are normal myeloid cells. In patients with abnormalities, the model number tends to be hyperploid (47-56). These clonal abnormalities are observed only in accelerated phase of the disease and in stable case it may not be present.

Consistent with the probable B cell origin of multiple myeloma 14q+ chromosome appears to be common to this disease (Wuster Hill et al, 1973; Philip et al, 1975 and Liang et al, 1979). In addition to 14 and 11 chromosome numbers 1, 6 and 8 are also involved frequently.