ABNORMALITIES OF SEX CHROMATIN

with particular reference to the triple-X
and chromatin-positive Klinefelter's syndromes
in mental defectives.

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"Barr has thrown a large stone in the pond and the ripples are still widening".

The 'Lancet', 1954.

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CONTENTS

PREFACE

Preface

In 1949 Barr and Bertram made the far-reaching observation that a sex difference could be readily identified in the intermitotic nuclei of the cat by means of an easily recognisable small chromatin body in the female nucleus. During the subsequent fifteen years a completely new field of research has opened up.

Within a few years of this initial observation, it was noted that sexual dimorphism could similarly be demonstrated in man. Since then, techniques for demonstrating this sex chromatin body have been applied to several fields of research in man, the most fruitful of which has been the investigation of the intersex states.

During the earlier years of application of this new technique, several discrepancies of nuclear sex in such intersex conditions were revealed. However, the precise nature of the sex chromatin body and its relationship to the X chromosome were not known, and workers could only speculate about the chromosomal sex status in these anomalies.

Although human chromosomes were first studied as early as 1879, progress in this sphere of research has been extremely

slow until very recently. Indeed, chromosome analysis in man has been a practicable and reliable diagnostic investigation for only the past five or six years. However, since 1959, following the introduction of vastly improved techniques, there has been a great volume of research into chromosome abnormalities in man. These have revealed numerous anomalies of both autosomes and sex chromosomes.

The technique of nuclear sexing has been shown to be an extremely useful rapid method of detecting whether certain abnormalities of the X chromosomes are present. Because of ease and rapidity of examination, the method has proved to be of particular value in mass surveys of large populations.

One such population which has been discovered to be a fruitful source of anomalies of sex chromatin and the sex chromosomes is that of mental defectives. It is towards abnormalities in this group that my researches have been directed and with which this thesis is largely concerned.

PART 1

HUMAN CHROMOSOMES

Human Chromosomes

Historical Background

The examination of human chromosomes has interested investigators for many decades, and, indeed, its history goes back for over 80 years, beginning with Arnold, who in 1879, first published drawings of chromosomes in human tumour cells. These pictures, however, are not really clear enough to give even a rough estimate of the number or type of chromosomes present. Three years later, Flemming also described and drew chromosomes in dividing corneal cells, but the first to attempt to actually count the number of human chromosomes was Hansemann, who, in 1891, using sections of testicular tissue, reported 18, 24 and over 40 chromosomes in 3 dividing cells. Painter, in an article in 1923, describes work done by several authors in the years following Hansemann's paper, and comments that conflicting results were given by these workers, who reported chromosome numbers of between 16 and 48.

In 1912, de Winiwater, who was the first to use fresh testicular tissue, claimed that, in metaphase in spermatogonia, he could identify a total of 47 chromosomes consisting of 23 autosomal bivalents together with an unpaired X chromosome. He also reported that 48 chromosomes were present in ovarian

tissue, and came to the conclusion that man belonged to the XX-XO type of sex determination.

Nine years later, in 1921, Painter, also using fresh testicular tissue, disagreed with de Winiwater both about the sex chromosomes and about the total number of chromosomes present in the male. Painter reported that, in the male, a small Y chromosome was present in addition to the X, and consequently concluded that man belonged to the XX-XY as opposed to the XX-XO sex chromosome type. It is interesting to note that, although at this time, Painter stated that "in the clearest equatorial plates so far studied, 46 was the most frequently observed number", 2 years later he revised this opinion, and stated that the normal diploid number was 48 in both sexes.

For the following two decades, there was great controversy about this question of the number of chromosomes in the male, and the nature of the sex chromosomes in primary spermatocytes. Most workers supported Painter, but de Winiwater and other workers such as Oguma and Kihara (1923) still claimed that 47 was the correct number of chromosomes in the male, and de Winiwater and Oguma (1926) reiterated their belief that there was only the single X sex chromosome in spermatocytes.

Investigation of human chromsomes then remained almost static for the next twenty years, and King and Beams in 1936 went so far as to say, "Human cytology is so far advanced as regards chromosome number in the germ cells that very little remains for research or discussion." !

The accepted number of chromosomes remained at 48 until nearly 8 years ago, when, in 1956, Tjio and Levan described in their classical paper how, by using techniques which were greatly improved compared with those of the earlier workers, they prepared and examined tissue cultures of lungs obtained from 4 legal abortions. Much to their surprise, the majority of fibroblast-like cells they observed contained 46, and not the accepted number of 48 chromosomes. Their ability to make this accurate observation was undoubtedly due to the fact that their preparations were vastly superior to any which had been previously published, and, indeed, were of such quality that the authors were even able to describe some of the more minute details of the chromosomes themselves.

This figure of 46 was rapidly confirmed by Ford and Hamerton (1956a) in squash preparations of testicular tissue, and has since been reported also by numerous other workers such as Chu and Giles (1959), Hsu and his colleagues (1957), Bender (1957), Ford et al. (1958), Tjio and Puck (1958a).

However, chromosome counts other than 46 have been reported by Kodani (1958), who examined squash preparations of testes of 15 Japanese and 8 white men. Although he agreed that 46 was the basic diploid number, in 7 of the 23 people he examined, he claimed to have observed 1 or 2 extra chromosomes which he considered to be similar to the small, inert and often heterochromatic supernumary chromosomes found in many species of insects, flatworms and plants. The reports of Kodani have suggested that the frequencies of individuals having 47 and 48 chromosomes are considerably higher in Japanese than in white people, but more recently Makino and Sasaki (1961) have found the normal number of 46 in aborted foetuses from Japanese mothers so that Kodani's observations must be considered suspect.

Makino and Sasaki (1961) also cite in their paper, the work of Chang who is the only other worker besides Kodani who has disagreed in any way with the accepted figure of 46. Chang, in 1959, using sectioned material from a female Chinese foetus, concluded that the oogonia contained 48 chromosomes, but Makino and Sasaki comment that since his counts were based on sectioned material in which the chromosomes were clumped together, his results were probably incorrect. There have been no further reports to substantiate the observations of Kodani or Chang, and in view of innumerable reports and photographs, 46 remains

the generally accepted figure.

The major factor enabling the correct number of human chromosomes to be established in 1956 was the vast improvement in technique which had taken place by this time. Because of the numerous chromosomal divisions taking place in the germ cells of the testis, this organ appeared, to the early investigators, to be the most suitable for examining chromosomes, and so the greater part of their work was done on testicular tissue which was fixed, paraffin embedded, and stained in the usual manner. In most cases, the tissue was obtained some time after death, often from executed criminals. The resulting appearances were difficult to interpret and the chromosomes difficult to count because of several factors, including the degenerative changes which occur very rapidly in the testis, and the artefacts which may arise very readily in the technique itself. In addition, in these histological sections, the chromosomes are crowded together, bend and overlap each other, with the result that an accurate count is extremely difficult to obtain.

Both de Winiwater (1912) and Painter (1923), used fresh testicular tissue in order to obtain a clearer demonstration of the chromosomes. This point was emphasised by Painter, and, realising its importance, Evans and Swezy (1929), working in America, waited literally at the foot of the gallows to obtain

testicular tissue from executed criminals, so that, for some of their preparations, this tissue could be fixed within one minute of death.

Nevertheless, even with these precautions, results were still not satisfactory, and visualisation of the chromosomes was not clear. Techniques gradually improved over the years, and the tissue culture method which is now the principal one used for chromosomal examination, slowly developed. Hsu (1952) and Chu (1960), give several reasons for the suitability of examining chromosomes by this method, and Hsu also quotes some observations made on this point by Fischer (1946), who was an earlier worker in the tissue culture field. These authors comment that cells in tissue culture grow best in monolayers, which, by their nature present the best conditions for direct observation and photography, and they add that since these cells are already flattened and stretched in the culture they are in a more suitable state for examination. They also make the further points that mitotic activity, which is greater in vitro, can be enhanced by experimental means, giving better visualisation, and that fixation of the chromosomes in tissue culture is immediate and more satisfactory than fixation of chromosomes in solid tissues. Finally, by avoiding any necessity for sectioning tissues, no material is lost or added by the

microtome knife.

Tissue culture methods for the examination of human chromosomes, had, in fact been used as far back as 1929, by Kemp, who had cultured embryonic liver, heart and spleen; it was not, however, for many years that this approach to the problem was revived. Indeed, it was not until 1952 that Hsu reawakened interest in this technique by describing chromosomes in cultures of embryonic skin and spleen to which he had accidentally added hypotonic saline, thus causing the chromosomes to spread widely apart and consequently become more clearly visible.

The tissue culture method was quickly seen to have obvious possibilities, and more workers became interested in this particular technique for examining chromosomes. As it gained popularity, further refinements of technique were added, most of which aimed at the all important point of spreading the chromosomes as far apart as possible. In addition to the use of hypotonic saline, two further improvements which were introduced, were the adoption of the "squash" method of making a slide preparation, and the use of colchicine. The "squash" method (Feulgen squash following acetic alcohol fixation), which was introduced by Sachs in 1952, incorporates mechanical pressure

for squashing and separating the chromosomes on the slide, but has now largely been superceded by the simpler air-drying technique for spreading the chromosomes, as described by Rothfels and Siminovitch (1958), and Tjio and Puck (1958b). Colchicine, which was introduced into the technique by Ford and Hamerton (1956b), had already been used in plant cytology for improving chromosome dispersion, and acts by breaking the spindle and producing arrest of the chromosomes at the stage of metaphase, thus leading to the accumulation of mitoses. It also causes chromosome constriction, thickening and shortening, together with wide separation of the arms of the chromosomes.

Long term tissue culture methods using various tissues such as skin or fascia are widely in use, and several workers such as Puck et al. (1958), Chu and Giles, (1959), Fraccaro et al. (1960b), and Harnden (1960), have described methods for initiating skin biopsy cultures. However, Harnden (1960) has pointed out that the use of long term tissue culture introduces the possibility that chromosome numbers in cells may alter after long periods of culture, the most usual changes in culture being an increase in the percentages of polyploid cells in subculture (Rothfels and Siminovitch 1958, Fraccaro et al. 1960b), and translocation or fragmentations of chromosomes (Hirschhorn and Cooper, 1961). Although Harnden (1960) found that abnormalities

such as polyploidy and endoreduplication occurred after 2 to 3 weeks of culture, other workers, e.g. Puck (1958), Tjio and Puck (1958), and Chu and Giles (1959) have maintained that under strictly controlled conditions, human cells can be grown successfully in tissue culture for long periods of time of up to several months.

The possible disadvantages of long term tissue culture have been obviated by the introduction of short term tissue culture methods employing cells from bone marrow and blood. These methods have the obvious advantages of the greater ease of repeating specimens and increased speed in obtaining results. However, although long term culture techniques have now largely been superceded by the shorter methods, as Harnden (1960) observes, long term tissue culture methods may still be profitably employed in the examination of several different types of tissues from subjects in whom a mosaic chromosomal abnormality is suspected, i.e. in individuals whose body contains two or more lines of cells with different chromosome constitutions.

The short term technique of cultivating human bone marrow for the purpose of examining chromosomes was introduced by Ford and his colleagues (1958), who combined Lajtha's suspension culture method for bone marrow cells (1952), with

a technique for processing bone marrow cells in the mouse, previously described by Ford and Hamerton (1956b).

Even the relatively minor discomfort of a bone marrow biopsy is no longer necessary. Nowell and his colleagues in 1958, introduced into this sphere of research a short term culture method employing peripheral blood. This method was based on the "gradient culture" principle of Osgood and his associates who had evolved a method of growing peripheral blood in tissue culture for other purposes. (Osgood and Krippaehne 1955, Osgood and Brooke 1955).

Nowell's method was later modified by Hungerford and his colleagues in 1959, when theyincorporated the use of phythaemagglutinin into the technique. Phythaemagglutinin, according to Rigas and Osgood (1955), is a mucoprotein extracted from Phaseolus vulgaris, or the French bean, and is used to agglutinate red cells and so separate out the leucocytes. Nowell (1960) has ascribed to this substance the most interesting property of initiating mitotic activity by modifying the cultured leucocytes into a state in which they are capable of division, so that mitotic activity takes place in vitro after a "long latent period" of 3 days after the addition of the phythaemagglutinin. During this time the cells "switch over" from their usual functions to the synthesis of protein and other materials necessary

for division.

For the following reasons the type of dividing cells in these peripheral blood cultures were considered by Hungerford and his colleagues (1959) to consist of monocytes together with large and medium sized lymphocytes. Firstly, they found that when fresh serum is added and mitoses cease, the entire population of cells differentiates into monocytes, macrophages or multinucleate giant forms, and no cells resembling mature granulocytes or small lymphocytes are observed. Secondly, autoradiographic studies have been performed in vitro by Bond and his associates (1958); in these, tritium labelled thymidine is incorporated into human leucocytes. Tritium labelled thymidine is a specific precursor of deoxyribonucleic acid (D.N.A.), and after its incorporation into the leucocytes, it is found only in the monocytes, and large and medium sized lymphocytes. These cells therefore must have the capacity to synthesise new D.N.A., and are presumably capable of division.

In addition to other techniques described by Clarke in her review (1962), Hungerford's method, with minor modifications added by some individual workers, is now widely used for the examination of human chromosomes. The standard of the results obtained can be extremely high, and, in favourable circumstances,

exceptionally clear preparations may be obtained.

Since the introduction of these rapid simple techniques, a large amount of work has been done, especially since 1956, increasing in volume each year, yielding much information about chromosome morphology together with abnormalities of sex chromosomes and autosomes in various diseases and syndromes. Although, in recent years, the results of a great number of detailed investigations have been reported, one must, nevertheless, only admire the pioneers in this field, including workers such as de Winiwater, Painter and Kemp, to mention only three, who, in spite of the limited techniques at their disposal, were still able to discern so much about chromosome morphology, and who spent so many hours of diligent research in this difficult task. One sympathises with Painter's cri de coeur - "J'ai perdu un temps énorme à répéter des numérations très fatigantes et j'avou aussie, très fastidieuses".

The Normal Human Karyotype

By using these improved techniques, which are described in detail by Clarke (1962), in her review, chromosomes may readily be observed in dividing cells when the process of division has been arrested by colchicine at the stage of metaphase. This is the stage of mitosis when the nuclear membrane has disappeared, and

the shortened and thickened chromosomes have clustered together on the equatorial plane of the cell, attached by their centromeres to the spindle which is composed of fibres radiating out in the cytoplasm from two centrioles situated at opposite poles of the cell. The chromosomes at this stage have divided longitudinally but have not yet separated, and the object of the techniques used in making preparations aims at evenly spreading the chromosomes as far apart in the cell as possible, diverging the two chromatids of each chromosome, and arranging the chromosomes in a suitable plane for microscopy.

According to Ford (1962) in his review of the subject, the chromosomes when examined in metaphase may range in length from about 7μ to 1.4μ. The number of chromosomes, as I have previously described, is now firmly established as 46, and by pairing these chromosomes and arranging them in order, these 46 chromosomes can be shown to consist of 22 pairs of autosomes together with the 2 sex chromosomes, XX in the female and XY in the male. (Fig. 1)

The chromosomes are paired and arranged in order by comparing their relative length and the position of the centromere , this position being expressed as the ratio of the length of the shorter of the two arms to the total length of the whole chromosome.

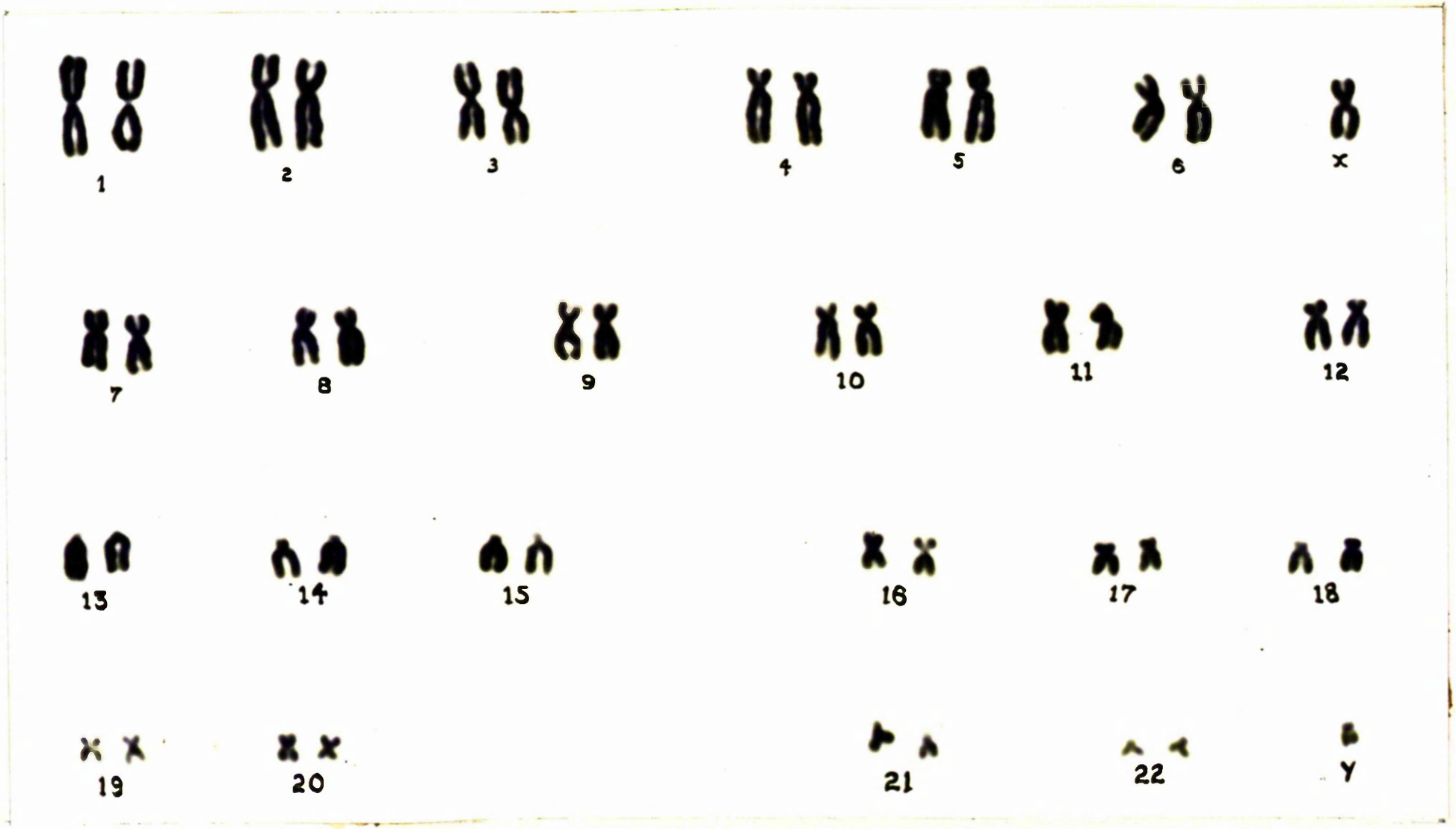

Figure 1 - Karyotype of a normal male showing the 22 pairs of autosomes together with the X and Y chromosomes, numbered according to the "Denver" international system (1960). Preparation made in the Pathology Department, Western Infirmary, Glasgow.

Even when the chromosomes have been paired as accurately as possible, Carr and his associates (1961a) note that there is frequently a difference of up to 15% in the length of homologous chromosomes, caused by factors such as differences in the degree of contraction of the 2 chromosomes, excessive mechanical extension of one of them on the slide, or foreshortening of a chromosome by a bend along its length in the plane of the optical axis of the microscope.

Various groups of workers such as Ford et al. (1958), Tjio and Puck (1958a), Chu and Giles (1959), Levan and Hsu (1959) and Patau (1960), have arranged the paired chromosomes into groups and have attempted to identify individual chromosomes. These workers however, adopted slightly different systems of grouping and numbering the chromosomes, and, in order to unify these various systems and prevent confusion, a conference group composed of investigators who had already published work on this subject, met in April, 1960, in Denver, Colorado, to prepare what is now known as the "Denver" system of classification which is now universally used.

The Denver study group agreed that after arranging the 22 pairs of autosomes in descending order of size, the autosomes could be classified into 7 groups, i.e. groups 1 - 3, 4 - 5,

6 - 12, 13 - 15, 16 - 18, 19 - 20, and 21 - 22. Each of these groups was described with particular reference to the length of the chromosomes and the positions of the centromeres; in the large 6 - 12 group it was noted that the greatest difficulty in identification of individual chromosomes occurred. The Y chromosome is similar to those of group 21 -22. The X chromosome is similar to the medium sized chromosomes with submedian centromeres in group 6 - 12, resembling, according to the report, chromosome number 6.

Although the Denver group states that "with very favourable preparations distinction can be made between most, if not all chromosomes", there has been some disagreement on this point, especially with regard to the 6 - 12 group. For example, whereas Patau (1960, 1961) has questioned the possibility of identifying any of the members of this group, Ford (1962) claims that it is possible to recognise the individual chromosomes in favourable preparations, and gives a description of each pair.

The Denver system has evoked some criticism, e.g. by Patau (1961) but nevertheless is now universally used, although in certain circumstances the system suggested by Patau, (1960), in which the 7 chromosome groups are designated by the letters A to G, is also used by various authors.

Certain chromosomes, or groups of chromosomes are described as being identified by the presence of 'secondary constrictions' and 'satellites'. Whereas the primary constriction of a chromosome is the centromere, the term 'secondary' is applied to any other constriction which may be present. Secondary constrictions occur most frequently at the ends of the chromosomes, with the resulting formation of satellites which are small apparently spherical bodies attached to the end of a short arm of a chromosome by a thin faintly staining stalk or 'SAT' zone. This SAT zone lacks readily stainable material (sine acid thymonucleic) and is thought to act as a nucleolus organiser during telophase (Carr and Barr 1961b). Satellites have been described by various groups of workers, e.g. Tjio and Levan (1956), Tjio and Puck (1958a), Levan and Hsu (1959), Chu and Giles (1959), and prominence was given to this feature by the Denver group who agreed that in all, 3 pairs of chromosomes, numbers 13, 14 and 21, were 'satellited'. The Denver report also stated that chromosomes 13 and 14 could be distinguished by the size of these satellites, for whereas the satellites on chromosome 13 were described as being prominent, those attached to chromosome 14 were small.

However, in 1961, Ferguson-Smith and Handmaker made the

further observation that satellites were present in all five acrocentric chromosomes, i.e. numbers 13, 14, 15, 21 and 22. In addition they gave details of the interesting phenomenon (also noted by Harnden 1961 and Petersen and Therkelsen, 1961) of satellite association, when the satellited chromosomes tend to adhere together during mitosis by their short arms. This association is thought to be related to the formation of nucleolar material in the region of the satellites.

As a result of this work on satellited chromosomes, it is no longer thought that individual chromosomes in groups 13 - 15 or 21 - 22 can be identified by the presence, absence or size, of satellites.

Apart from the secondary constrictions associated with satellite formation, other secondary constrictions have been noted by, e.g. Patau (1961), de la Chapelle (1961), Muldal and Ockey (1961, 1962) and Ferguson-Smith et al. (1962). The most recent of these reports (Ferguson-Smith et al. 1962) presents an idiogram showing sites of up to 20 secondary constrictions some of which appear infrequently, and the authors claim that in at least 15 sites these constrictions are characteristic of the chromosome concerned.

Chromosome abnormalities in cell division

Although, as I have just described, the great majority of individuals possess a normal chromosomal karyotype, a large number of chromosomal abnormalities have been reported involving both the sex chromosomes and the autosomes. These structural or numerical abnormalities, which may involve either the whole or part of a chromosome, arise during the complex procedure of cell division, either in meiosis or mitosis.

During the normal first meiotic division of a germ cell, the 44 autosomes together with the 2 sex chromosomes forming the diploid number of chromosomes, divide to form 2 cells, each containing 22 autosomes together with 1 of the sex chromosomes, thus, in this reduction division, forming the haploid number of chromosomes. In the normal second meiotic division, each of these 2 cells follows the pattern of a mitotic division, resulting in 4 cells produced from the single precursor cell. Early in the first meiotic division, while the homologous chromosomes come together and form bivalents, "crossing over" of genetic material occurs between the homologous chromosomes at the chiasmata at which the 2 chromosomes are held together. Structural abnormalities of the chromosomes probably take place most frequently when this "crossing over" occurs, for during this

process, the chromosomes must break and rejoin, and are liable to rejoin incorrectly with the consequent formation of various abnormal forms. The structural abnormalities, which may be produced in this way, are described by Harnden (1962), as being inversion, deletion, duplication, translocation, or formation of isochromosomes.

Inversion is said to occur when part of a chromosome becomes inverted so that the genes involved come to lie in the reverse order to that originally present. According to Harnden (1962), the expression of the genes may be affected by their position on the chromosome, and he states that "conclusive proof that an inversion had occurred could probably only be obtained from independent genetic observations and it is unlikely that one could get such evidence in man".

Deletions occur, when, during the "crossing over", involving the breaking and rejoining of the chromosomes, the broken ends incorrectly unite, due to perhaps lying in a looped position, so that part of a chromosome becomes eliminated. A special type of deletion of a chromosome may occur giving rise to a 'ring' structure. According to Lindsten and Tillinger (1962), ring chromosomes have occasionally been observed in malignant tumours and in single cells after therapeutic

irradiation, and they also add that these ring chromosomes are unstable, being liable to spontaneous, numerical and structural change.

The third type of morphological abnormality is translocation, in which part of a chromosome is transferred to a different site on the same chromosome, or is transferred to a completely different chromosome. The majority of translocation abnormalities reported in man have involved chromosomes in group 13 to 15 or 21 and 22, which as I have previously mentioned, bear satellites. It is thought (Polani et al 1960), that the stalk which attaches the satellite to the main chromosome body is concerned with the formation of nucleolar material, and Ohno and his colleagues (1961), have demonstrated how this mechanism may be connected with the phenomenon of translocation. They have suggested that during the formation of a nucleolus, the stalk, or nucleolus organising part of the chromosome becomes greatly stretched and is consequently liable to breakage. They further conclude that the close proximity of these acrocentric satellited chromosomes, or the 'satellite association', described by Ferguson-Smith and Handmaker (1961), would then facilitate the formation of such structural abnormalities as translocations between the various members of this group.

The fourth type of morphological abnormality which may occur during cell division is the formation of an isochromosome. This structure arises by a chromosome, at the beginning of anaphase, splitting transversely instead of longitudinally, with the resulting formation of a perfectly metacentric chromosome which has completely homologous arms united at the centromere.

Ford (1962) observes that these structural changes appear spontaneously in the exceptional individual, but may be readily induced by irradiation or exposure to chemical mutagens, many of these abnormalities being incapable of regular perpetration at mitosis.

In addition to these abnormalities involving structural changes in part of a chromosome, numerical abnormalities may also arise involving the whole chromosome. These abnormalities are believed to be due to the process of non-disjunction occurring either in meiosis or mitosis and in the germ cells, for example, arise when for some reason, a pair of homologous chromosomes fail to separate during either the first or second meiotic division. Non-disjunction affecting the sex chromosomes can be much more complicated than when non-disjunction involves the autosomes, largely because the sex chromosomes in the male are unlike.

Consequently, in theory, non-disjunction can lead to many different combinations of the X and Y chromosomes in the resulting gametes and zygotes. Some of these sex chromosome abnormalities and a few autosomal trisomic conditions have been described in certain syndromes, but there is no doubt that many chromosomal abnormalities resulting from non-disjunction are lethal.

Apart from occurring in the stage of meiosis, it is believed that non-disjunction may arise in the zygote, its occurrence in mitosis during early embryonic life giving rise to daughter cells each with a different number of chromosomes, thus forming a mosaic, which, by definition, is an "individual whose body contains two or more readily demonstrable lines of cells with different chromosomal constitutions which have arisen within a single individual". (Harnden 1962). For example, if non-disjunction arises in the first mitotic division of a zygote, a mosaic with two lines of cells will be formed. A similar sort of error may also arise because of "anaphase lagging", when one chromosome fails to move at anaphase in mitosis, with the result that this chromosome is either included in the wrong cell or is eliminated altogether, (Harnden, 1962).

Many of these chromosome anomalies just described are

illustrated in greater detail in later parts of this thesis when I discuss the various aberrations of chromosomes, especially of the sex chromosomes, which have been revealed by nuclear sexing techniques.

I now turn to the historical background and to the development of these nuclear sexing techniques, which in their application to man have been of great value in yielding information about the normal and abnormal structure and functions of the sex chromosomes.

PART 2

SEX CHROMATIN

Sex Chromatin

Historical Background

Although the earlier investigators in the field of chromosome research had been studying the details of the chromatin structure of the human cell both in meiosis and in mitosis, and had recognised and described the XX and XY pairs of sex chromosomes, they had not realised that a sex difference could, in fact, also be readily observed in the majority of resting intermitotic nuclei. Indeed, it was not until 1949, that Barr and Bertram, who, at that time, were working on an entirely different problem, first observed the sex chromatin body, a structure, which, as Lennox (1956b) remarked in his review of nuclear sexing, had "lain under the noses of microscopists for about 70 years, ever since oil-immersion observation of histological sections became a satisfactory procedure".

The events leading to the recognition of sex chromatin are related by Barr (1958). He describes how Hodge, around 1890, had reported structural changes occurring in the nerve cells of bees and swallows after prolonged periods of work and flight. Since then, much work had been done on the effects of altered levels of activity on the neuronal state. In 1949, Barr and

Bertram were working on a problem involving fatigue in air-crews, and, in order to do this, were studying the effects of fatigue in nerve cells of cats. After fatigue was produced by stimulating the hypoglossal nerve, the cats were kept alive for varying periods of time, and the structure of the neurons of the hypoglossal nerve was then observed in histological sections stained with cresyl echt violet. While Barr and Bertram were observing the nuclei of these nerve cells, they noticed that after stimulation of the hypoglossal nerve, a small mass of chromatin called the 'nucleolar satellite', which was present in 30 to 40% of cells, enlarged slightly during the period of dissolution of the Nissl bodies, and moved away from its most frequently encountered position adjacent to the nucleolus. This satellite moved for varying distances towards the nuclear membrane, and then returned to its former position when the cell became normal again. The nucleolar satellite was noted to be present in some, but not all of the cats, and Bertram, who at that time was a graduate Science student, made the significant observation that, in fact, the satellite was present in female cats only. It was noted at the same time that in male cats also, a much smaller satellite was present in a few nerve cells, situated adjacent to the nucleolus. This structure

was near the limits of resolution of the oil-immersion objective. Later work by Barr and his colleagues, (Barr and Bertram 1951, Barr et al 1950), showed that after stimulation of the hypoglossal nerve of male cats, these very small nucleolar satellites also increased slightly in size, and their incidence rose a little.

Barr and his co-workers (1950) suggested that the nucleolar satellite they had observed in the female was related to two X chromosomes, and according to Moore (1962a) they "considered using the term "X-chromatin" but decided against it because the origin of the chromatin mass could not definitely be traced to the chromatin (heterochromatin) of the X chromosomes". Instead, therefore, the term "sex chromatin" was given to the nucleolar satellite, and as such, it is now universally known.

However, other investigators did not agree with Barr's work, opposition being given by both Coidan (1952) and Brusa (1952). Coidan, after examining several species of animals, including the cat, rat, mouse, gorilla, vole and man, denied altogether that sex could be differentiated by means of this chromatin body. Brusa on the other hand confirmed Barr's observations in cat nerve cells, but after finding a chromatin body in both sexes in the pigeon, came to the conclusion that

Barr's explanation of this body was unlikely. Since then, innumerable observations by many investigators have confirmed Barr's original belief that the nucleolar satellite is related to sex, and in particular to the X chromosome.

In the mean-time, however, it was being established that sex chromatin was more widely distributed in the body, and was present in the cat in tissues other than neurons. In 1951, Barr reported that it was present in the neuroglial cells, and in 1952, Graham and Barr showed that most of the cells of the cat could be sexed. Other species besides the cat were examined (Moore and Barr, 1953), and then in 1953, Moore and his colleagues made the important observation that in man, also, it was possible to detect sex in skin biopsies. This observation was rapidly confirmed by other workers such as Hunter and Lennox (1954) and Emery and McMillan (1954), and interest in the procedure became generally aroused.

This method of detecting sex chromatin in skin biopsies was used until 1955(a), when Moore and Barr described a simpler method of detecting the chromatin body in smears of cells from the oral mucosa. Since then, using these and other techniques of examining nuclear sex, there has been a great deal of research into various abnormalities involving the sex chromatin body, and

this research combined with chromosome investigations has helped to elucidate a few of the problems concerning the sex chromosomes in man.

Description of Sex Chromatin

In the first animal cells to be studied, namely, the cat neurons, the sex chromatin body is most frequently situated adjacent to the nucleolus (Barr and Bertram 1949), but it may also be found free in the cytoplasm or occasionally, lying against the nuclear membrane (Barr and Bertram, 1951). The position of sex chromatin in nerve cells varies between species. For example, in man (Mylle and Graham 1954), and in the rhesus monkey (Prince et al 1955), the sex chromatin, unlike that of the cat, is more commonly found in the peripheral position in nerve nuclei. Indeed, in most female tissues, the sex chromatin body is found more frequently in the peripheral position, forming a mass lying against the inner part of the nuclear membrane, sometimes, apparently, according to Klinger (1962), on an indentation in the membrane, and in long slender nuclei, such as smooth muscle nuclei, the chromatin body may be observed at the tip. Sex chromatin can also lie free in the cytoplasm in man, but in this position, as Klinger (1962) points out, it is difficult to

distinguish from other non-sex chromatin bodies which may be present, especially in nuclei of the oral mucosa, which is the tissue most commonly employed for nuclear sexing. For this reason, therefore, only chromatin bodies attached to the nuclear membrane can be safely regarded as sex chromatin.

Little is known about the cause of the variations of the position of sex chromatin in the cell. In his review, Barr (1963) describes Graham's observations of the movements of the sex chromatin body in cells of cat embryos. In neuroblasts, during development, sex chromatin moves from the nuclear membrane to the juxta-nucleolar position, whereas in non-nervous tissue, a less pronounced change occurs in the reverse direction, the sex chromatin body moving from the juxta-nucleolar position to the nuclear membrane. These observations, together with the movements of the sex chromatin body of nerve cells during chromatolysis in the cat, suggests, according to Moore (1962c) and Barr (1963) that the position in the cell may depend on certain metabolic factors.

Sex chromatin is described by Klinger (1962) as being unusually plano-convex in profile, and sometimes spherical, triangular, "V" shaped, or dumb-bell shaped. In smears from the oral mucosa it may be flattened against the nuclear membrane,

but when lying free in the cytoplasm, Barr (1963) notes that it may adopt a spherical form. Occasionally, as was originally described by Graham and Barr (1952), and Crouch and Barr (1954), sex chromatin may appear as a bipartite structure.

In his review, Barr (1963) observes that the chromatin body is of the same order of size in different representatives of the mammalian class, but there may be minor variations between one cell type and another, being, for example, a little larger in adrenal cortex, thyroid epithelium and cartilage. Various average dimension have been quoted by various workers. For example, in man, sizes of 0.8 x 1.1μ, 0.7 x 1.2μ and 0.73 x 1.15μ have been described by Barr and Carr (1962b), Moore and Barr (1955a and b), and Sohval and Casselman (1961) respectively. The latter authors also make the point that, normally, the mean areas of sex chromatin bodies in the human, do not vary by more than 20 to 25% in the same person over long periods of time, and do not differ normally by more than 30 to 35% between different individuals.

The percentage of nuclei containing sex chromatin varies, as Moore (1962b) points out, according to the type of preparation, the technical quality of the specimen, the fixative, the staining technique, and the experience of the observer. Moore also notes

that the frequency can vary according to the source. For example, Moore and Barr (1953) state that sex chromatin can be identified in approximately 90% of nerve cells in females, and a figure even as high as 96% has been given by Graham (1954) in whole mounts of embryonic membrane.

Sex chromatin can be stained by numerous methods enumerated in reviews by Lennox (1956b) and Barr (1963). These authors list stains and methods such as Feulgen, haematoxylin/eosin, cresyl violet, thionin, methyl green pyronin, gallocyanin, fuchsin, Biebrich scarlet-fast green, and orcein. According to Barr (1963) it can be stained by a silver carbonate method but is refractory to other silver techniques. Sex chromatin also resists digestion after ribonuclease (Lennox 1956a) and persists after mild acid hydrolysis (Klinger and Ludwig 1957).

Sex Chromatin in the embryo

Graham, in 1954, was the first to show that sex can be determined in the intermitotic nuclei of the embryo before the gonads have differentiated, and Park later demonstrated in 1957 (a) that a reliable sex difference is established in the cells of the human and macaque embryo as early in foetal life as between the 12th and 19th day. Among the earliest embryos described are those which were examined by Park, and by Austin and Amoroso

(1957). Park examined human embryos as early as the 2 cell stage. Austin and Amoroso, working with cat embryos also examined 2 cell embryos, and nuclei of embryos of up to 20 days gestation, but in this material recognised sex chromatin in none of 26 morulae and in only 1 of 12 blastocysts. They suggested that the lack of sexual dimorphism in the early stages of development might be due to the fact that the nuclei of the embryo become smaller as the embryo develops, and that sex chromatin could only develop when the nuclei reach a sufficiently small size. However, more recent theories regarding the visibility of sex chromatin in the embryo around the 12th day have been put forward by Lyon in 1961, and will be discussed in part 6.

Although Witschi (1957) claims to have identified sex chromatin in the large vesicular nuclei of primordial germ cells of human embryos before the gonadal primordia are organised, sex chromatin has not been found in oogonia of human foetuses aged 3, 7 and 9 months examined by Ohno and Klinger (1962), and, according to Grumbach and Morishima (1962), has never been identified in the mature ovum. Observation of the chromosomes in the oogonia of human foetuses led Ohno and Klinger to the interesting conclusion that oogonia cease to propagate while the

female is in the 5th or 6th month of foetal life, and that several weeks before the end of gestation all the oocytes have completed the entire process of the first meiotic prophase, to remain suspended in a diffuse interphase-like dictyotene stage until just before they are shed at ovulation. A similar situation has been shown to exist in certain animals by several authors mentioned by Hamerton (1962) in his review.

Sex Chromatin in Animals

Since sex chromatin was first described in the cat, the investigation of many other animal species has shown that sex chromatin appears to be fairly widely distributed throughout the Animal Kingdom. Comprehensive reviews on this subject by Grumbach and Barr (1958), Ashley (1962), Moore (1962a) and Barr (1963) mention numerous workers in this field and detail their findings.

According to Moore (1962a), nuclei have been examined for sexual dimorphism in 35 mammalian species, together with 1 species of reptile, 1 kind of amphibian and several species of insects. Moore states that sexual dimorphism has been demonstrated in at least 26 mammalian species, but whereas no sex difference is present in reptiles or amphibia, there appears to be a

sexual dimorphism in the nuclei of certain invertebrates and in some cells of various birds.

In addition to the form of sexual dimorphism which is visible in the nuclei of tissue sections as the sex chromatin body, the other form of sexual dimorphism which is present in human neutrophils as "drumstick" structures, is also present in some other animals, and in their review, Davidson and Smith (1963) list several species in which this phenomenon has been noted. They observe that the frequency of these drumsticks is very variable, being scanty in some species such as cattle, and frequent in others such as the rabbit. Pedunculated nodules are present in both sexes in various rodents, but no sex difference has been recognised in the leucocytes of birds.

Sex Chromatin in Man

Sex chromatin is visible in many tissues in the human female, and is most clearly demonstrated, according to Lennox (1956b), in cells of the skin, squamous mucosa, nerve cells, adrenal cortex, cartilage and young granulation tissue. However, some cells, as Lennox points out, are difficult to sex, including for example, cells such as lymphocytes in which the nuclear structure is dense, cells which are coarsely granular such as

liver cells, or cells such as prostatic cells which often have small dense nucleoli somewhat resembling sex chromatin.

The typical sex chromatin body in the female is, on the whole, easy to recognise, and nuclear sex may readily be determined. However, as I have mentioned, when Barr and Bertram made their original observations on sex chromatin in the nerve cell nuclei of cats, they noted that a small chromatin body was also present in a few nerve cell nuclei in the male cat. In the human male also, a similar chromatin mass has been described in a few cells, sometimes, according to Sanderson and Stewart (1961), adopting the form of a crescent in nuclei of the oral mucosa. The incidence of this chromatin body in male cells is low. For example, Nelson (1956) states that in skin, 1 to 12 % of nuclei in the male contain this chromatin mass, and in the oral mucosa, 0 to 4% of nuclei. The chromatin body, however, is usually too small to be confused with the large sex chromatin body seen in the female, and Maclean et al (1961) go so far as to say that such bodies are never found in normal males.

Under certain circumstances, the incidence or size of sex chromatin, or the number of sex chromatin bodies present in one nucleus, may be altered. For instance, according to Barr and Carr (1962b), where varying degrees of polyploidy occur in tissues

such as the amnion, liver or bronchial mucosa, the number of sex chromatin bodies in the nuclei may increase, for example to 2, 3 or even 4. With regard to alteration of the incidence, Taylor (1963) has observed that newborn female infants show a low incidence of sex chromatin in nuclei of the oral mucosa, this effect being most marked during the first 2 days of life, when, she has noted, the sex chromatin body also appears larger and more diffuse than usual. Taylor states that certain hormones such as oestrogens, hydrocortisone or the adrenocorticotrophic hormone have been found to lower the incidence of sex chromatin, and suggests that the low incidence in the newborn may be a reflection of a metabolic change associated with neonatal adaptation, being possibly influenced, for example, by the high level of maternal oestrogens at this age. As far as alterations in size of the chromatin body are concerned, Sohval and Casselman (1961) have found that certain antibiotics administered both orally, and topically to the buccal mucosa, have induced a reduction in size of up to 65 % in mean area, the size returning to normal shortly after withdrawal of the drug. The prevalence of sex chromatin was not affected.

In addition to these physiological and iatrogenic causes, alterations in the size and incidence of sex chromatin, and in

the number of sex chromatin bodies visible in each nucleus, may also be associated with certain numerical and morphological abnormalities of the X chromosomes. These abnormalities will be described in the appropriate parts of this thesis which deal with certain abnormalities of the sex chromosomes in man; and the significance of the association between abnormalities in sex chromatin and the X chromosomes, which throws light upon the fundamental nature of the sex chromatin body itself, will be discussed in part 6.

Methods of detecting sex chromatin in the human

Several methods for the detection of sex chromatin in the human have been used, the first of which was the skin biopsy method introduced by Moore and his colleagues in 1953. According to Lennox, in his review in 1956(b), skin from any part of the body can be sexed, the sex chromatin body being best observed in fibroblasts, epidermal cells below the stratum granulosum and in the mouths of large sebaceous glands, (Fig. 2) Fixatives and staining methods vary. Moore and his associates originally stained the skin sections with haematoxylin/eosin, or by the Feulgen technique after fixing the tissue in a modified Davidson's fixative (a formol acetic-alcohol fixative). This

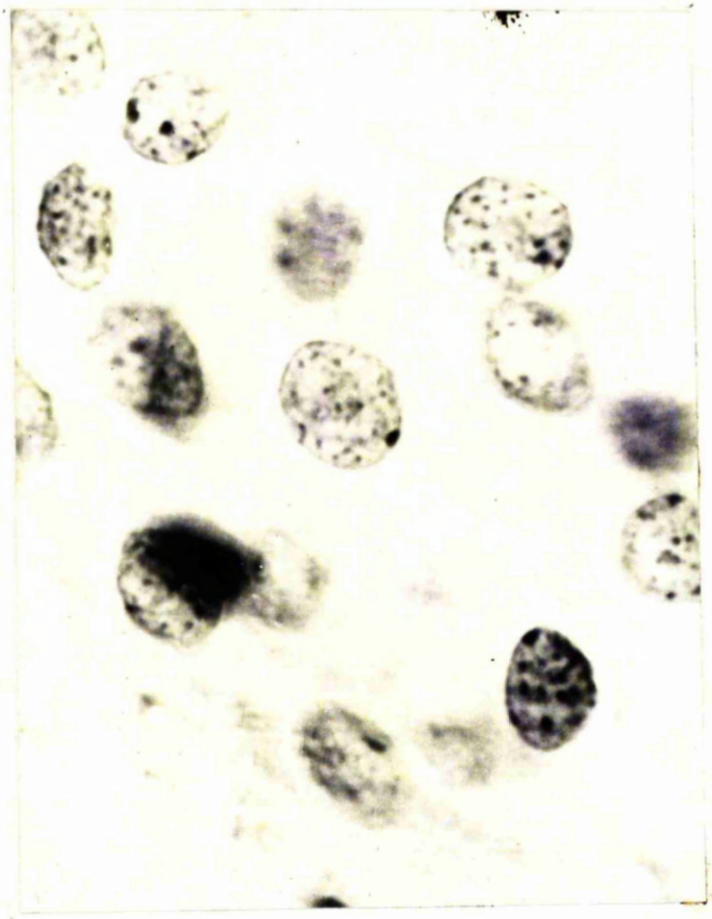

Figure 2 - Epidermal nuclei containing sex chromatin.
(Feulgen. X 1000).

fixative is still preferred by many workers, although according to Lennox (1956) other fixatives such as neutral formol or mercuric chloride may be used.

Various staining methods have been employed including many from those previously listed on page 37 . Preference is given by individual workers for different stains and modifications, amongst the most popular being Feulgen, haematoxylin/eosin, and thionin. It is generally agreed however, that whichever fixative or stain is employed, the preparations must be of high technical quality.

As previously noted, the percentage of nuclei recorded as containing sex chromatin may vary according to several factors, and, consequently, slightly different values are given by various workers. For example, to quote only 3 sets of figures, Moore and his colleagues,in their original description of the skin biopsy technique,give a figure of 52 to 85%, Barr (1960) gives a figure of 60 to 80%, and Emery and McMillan (1954) who rapidly confirmed Moore's finding of sex chromatin in the skin, give a much lower range of 25 to 54%.

Emery and McMillan also studied sex chromatin in post-mortem skin, and although they concluded that results are less accurate because of autolytic changes affecting the sex chromatin

the detection of sex by this technique can be of obvious importance in medico-legal work. In this connection, Dixon and Torr (1956, 1957) have found sex chromatin in cells of untreated limbs that had been exposed to air, buried, or exposed to water for several weeks, the chromatin body persisting for 2 to 3 weeks, depending on the nature of the environment.

However, for routine nuclear sexing purposes, the original skin biopsy technique has largely been replaced by the simpler method of examining nuclei in smears from the oral mucosa, (Fig. 3). This simple method was first described by Moore and Barr in 1955(a), and involves taking a smear from the inside of the mouth, placing the cells thus obtained on a slide which is fixed immediately and then stained. Again fixatives and stains vary slightly. The fixative most frequently used is Papanicolaou's fixative, (equal parts of 95% ethyl alcohol and ether), which was used in the original method described by Moore and Barr. Other fixatives have also been employed, such as methyl alcohol or acidified Papanicolaou's fixative, mentioned by Lennox (1956b) in his review, or modified Davidson's fixative as used by Marberger and her associates (1955). The staining method most frequently employed is again that described by Moore and Barr in their original report, namely cresyl echt violet, but the Feulgen stain

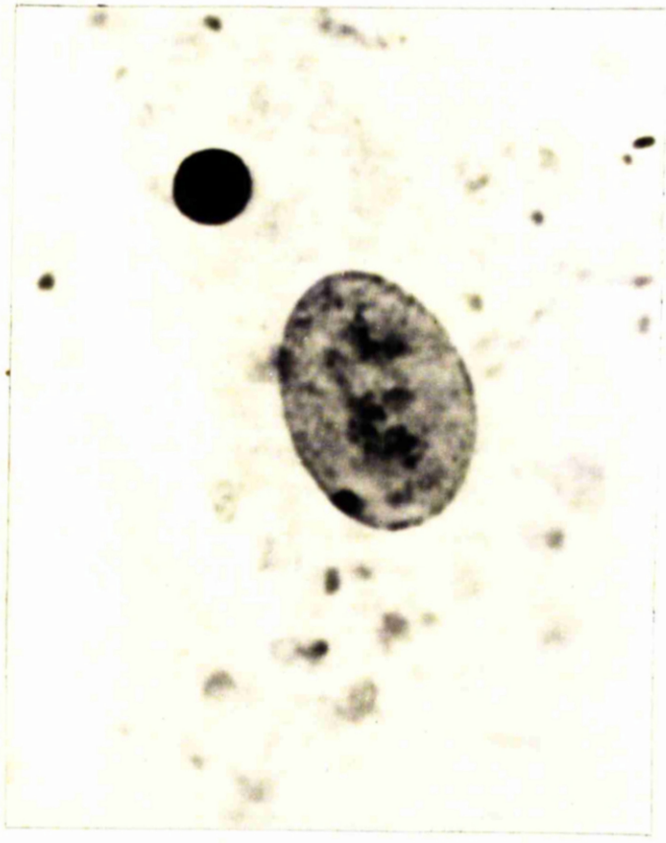

Figure 3 - Nucleus of an oral mucosal cell containing one sex chromatin body. (Cresyl echt violet. X 1,750).

has been used satisfactorily by Marberger and her colleagues (1955), and thionin by, for example, Graham and Barr (1952). Sanderson and Stewart (1961), and Thuline (1961) both advocate a technique incorporating aceto-orcein, the former authors describing a rapid method which is useful for immediate examination of buccal smears at the time of taking the specimens. A minor disadvantage to the oral mucosal smear method is that the presence of large numbers of bacteria may obscure the sex chromatin body, and in order to eliminate this factor, Klinger and Ludwig (1957) have recommended mild acid hydrolysis.

The percentage of nuclei stated to contain sex chromatin again varies according to different workers. For example, whereas Moore and Barr originally reported a range of 40 to 60%, figures of 20 to 79% have been given by Nelson (1956), 12 to 60% by Ridler and his colleagues (1963), and 36 to 76% by Maclean and his associates (1961).

The oral mucosal smear method of detecting sex chromatin is now the most widely used technique, being especially valuable in carrying out surveys of sex chromatin in large populations, for, as Moore and Barr pointed out, when they originally introduced it, the method is simple, further smears may easily be repeated, and in contrast to the skin biopsy technique, smear preparations are

easier to interpret, requiring less experience in cytology.

Apart from the oral mucosa, smears have also been prepared of cells from the vaginal mucosa by workers such as Carpentier et al (1955), Guard (1959) and Carpentier (1962). Carpentier, using Papanicolaou's fixative, and staining the cells with the Papanicolaou or Feulgen staining methods or with thionin, considers that although the buccal smear is easier to obtain, the prominence of the sex chromatin body and the fewer cytoplasmic inclusions makes the vaginal smear technique of value, good results being obtained even in the presence of oestrogenic influences which cause nuclear shrinkage and pyknosis.

In addition to examining vaginal smears, Carpentier and his associates have also prepared smears from the urethra, which, they claim, in spite of the presence of cytoplasmic inclusions are also suitable for sexing. Examination of cells from the urinary tract has also been performed by Eskelund who has claimed to have sexed epithelial cells in the urine.(1956).

At one time, it appeared possible that foretelling the sex of a foetus by examining cells in the amniotic fluid would become a popular practice, when several workers including Dewhurst, James, Fuchs and Riis, together with Sachs and his colleagues, independently, in 1956, reported the results of their investigations

in this field. With regard to the origin of these cells, James points out that material desquamated from the skin of the foetus consists of pyknotic debris, and considers that the sexable nuclei in the amniotic fluid probably have their origin in the vagina or urinary tract, or, as also suggested by Fuchs and Riis, the alimentary tract. Many of these investigations were made on amniotic fluid obtained near term either by trans-abdominal puncture, artificial rupture of the membranes, or at Caesarian section. However, fluid from earlier pregnancies has also been examined for example by Dewhurst, and by Sachs and his associates who have obtained amniotic fluid from an embryo as early as 8 weeks.

However, this technique of nuclear sexing has not been practised in this country, because, as Dewhurst pointed out, there were a few minor complications of labour and pregnancy in some of the cases examined, and it has been generally agreed that the small risk involved is not considered legitimate where merely curiosity is the reason for the investigation. According to Riis and Fuchs (1960), the theoretical risks to the mother include puncture of the bladder or bowel, or contamination of the uterus following uterine puncture, whereas in the case of the foetus, the risks include a possible resulting abortion. On the

other hand, Riis and Fuchs consider that these risks are very remote, and recently, in 1960, advocated this technique of nuclear sexing for the eugenic prevention of hereditary sex-linked recessive diseases such as haemophilia or sex-linked muscular dystrophy, for when such diseases were carried by the mother a therapeutic abortion of a male foetus would prevent the possibility of the disease being inherited. These workers, however, practice in Denmark, where the law regarding legal abortion differs from this country, and where legal abortion may be granted if "there is a close risk that the child, due to inherited characteristics or to disturbances or disease acquired during foetal life, may come to suffer from mental disease or deficiency, epilepsy, or severe and non-curable abnormality or physical disease".

In addition to being detected in vivo by the methods I have described, the sex chromatin body may also be demonstrated in vitro. For example, Serr and his associates (1958) have described sex chromatin in tissue culture preparations of human thyroid, and Miles (1959 , 1962) has reported sex chromatin in cultures of normal female tissues as well as in cultures of various benign and malignant tumours. This tissue culture technique of demonstrating sex chromatin, is, however, for obvious reasons,

not used for routine nuclear sexing purposes.

Apart from the usual form of sex chromatin which may be detected in various tissues by the preceding techniques, it is also possible to determine sex in neutrophils of the peripheral blood by means of a sex specific pedunculated nodule, first described by Davidson and Smith in 1954, and confirmed by numerous other workers cited by Davidson and Smith in their review in 1963.

In peripheral blood films, stained by any of the usual methods, this sex specific nodule, characteristic of the female, may be observed in polymorphonuclear leucocytes, or occasionally in eosinophils (Riis 1955) or basophils (Davidson 1962), (Fig.4). The nodule is described as presenting an appearance similar to a drumstick, composed of a small, defined, solid head of average diameter 1.4 to 1.6μ, which is joined by a single thread-like structure measuring 3μ in length to one lobe of the nucleus. According to Davidson and Flute (1962) a small 0.2μ diameter clear space is present near the base of the nodule.

It has been emphasised in the original report and in the more recent comprehensive reviews of Davidson and Flute (1962), and Davidson and Smith (1963), that this drumstick must be differentiated from other projections of the nucleus which may

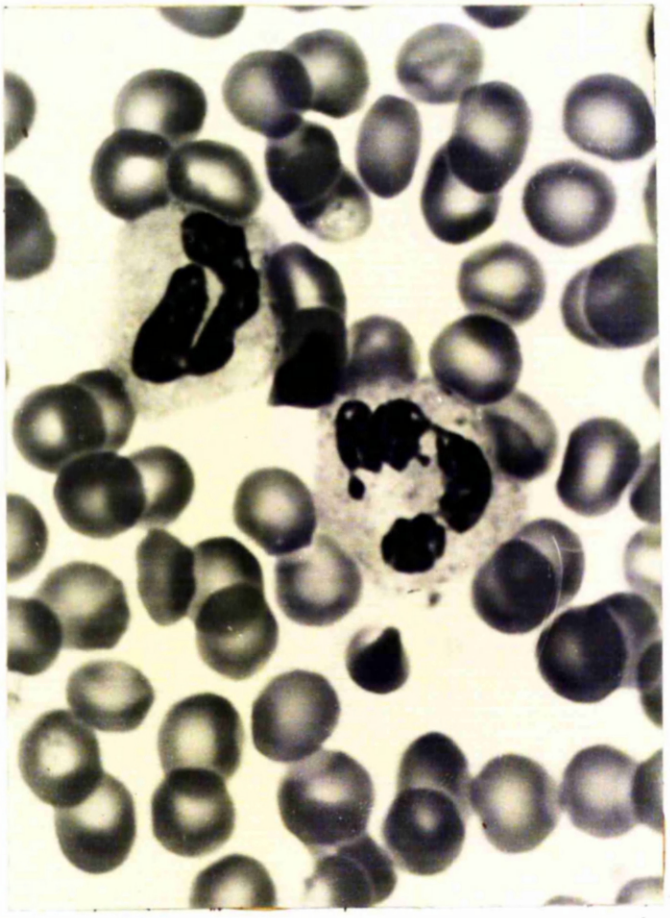

Figure 4 - Blood film from a normal female. The lower neutrophil shows a drumstick projecting from the upper lobe. A small club projects downwards from one of the middle lobes. (Leishman. X 1,500).

be present in both sexes, and with which the drumstick may be confused. Several types of projections are described. For instance, true drumsticks must be distinguished from sessile nodules which form blunt projections from the nuclei of neutrophil or eosinophil leucocytes and are of the same size as drumsticks. These sessile nodules are regarded as being strongly suggestive of female sex. A drumstick-like nodule may very occasionally be found in the neutrophils of males, but may be distinguished from true drumsticks by the fact that even if they are the correct size, they do not have the typical structure, and do not stain in the characteristic fashion. Other projections of the nucleus which must be differentiated from drumsticks are small clubs and the normally occurring terminal nuclear segments. Small clubs, which are often multiple and are commoner in the male, measure less than 1 μ in diameter or approximately one third of the size of sessile nodules.

As far as the frequency of drumsticks is concerned, in their original report of 1954, Davidson and Smith recommend that six drumsticks should be sought, which, in the female, should be easily found in 500 cells or in a frequency of 1%. They later state in their 1963 review that although on the average, 1 drumstick is found in 36 neutrophils, this number may vary considerably, for

example, from 1 drumstick in 5 neutrophils to 6 drumsticks in 2,000 neutrophils. They also mention the fact that the incidence of drumsticks in eosinophils is, on the average, 1 in 29, being slightly greater and less variable than in the neutrophils. In order to shorten the rather lengthy procedure of examining 500 cells, Davidson and Smith (1963) describe a screening test in which the frequency of both drumsticks and sessile nodules is noted in 50 neutrophils, and comment that in the female, even when drumsticks are few, sessile nodules may be found in reasonable numbers.

In certain circumstances, the frequency of drumsticks may be altered. For instance, Wiedemann(1958) describes an increased frequency of drumsticks at birth, especially in premature babies, but although his findings suggest that the frequency decreases from birth to adult life, Davidson and Smith (1963) do not consider that this latter observation has been adequately substantiated. The frequency of drumsticks may also vary according to the degree of segmentation of the nucleus, appearing less frequent when the neutrophil nucleus is less segmented than usual, and, conversely, more frequent when the neutrophil nucleus is more segmented than usual, for example, in the oversegmented neutrophils of untreated pernicious anaemia. (Davidson, 1962).

Alterations in the size of the drumstick nodule and in the number of drumsticks present in the neutrophils may also be associated with numerical and morphological abnormalities of the X chromosomes, and will be discussed, along with the corresponding changes visible in the sex chromatin body under similar circumstances, in part 6.

In practice, of all the techniques which have been described, the tissues which are commonly employed for studying sex chromatin in the human are the oral mucosa, skin and blood, the buccal mucosa being the most convenient tissue for routine nuclear sexing, especially of large populations, although the simultaneous study of sex chromatin in the skin, blood and other tissues may be of great value when dealing with individuals in whom there are certain abnormalities of sex chromatin and the X chromosomes.

Correlation between drumsticks and sex chromatin.

This is a convenient point at which to consider the correlation between drumsticks and sex chromatin, for the nature of the drumsticks has been the source of some degree of controversy. At the onset, Davidson and Smith suggested that the drumstick was a similar manifestation to the sex chromatin body in tissue cells,

consisting of the "female chromatin aggregate separated off as a drumstick-like appendage", but this opinion has encountered some opposition.

For example, it was suggested by Briggs and Kupperman in 1956 that drumstick frequency might be influenced in some way by the hormonal environment, but investigations by workers, e.g. Pfeifer (1962), and Ashley (1962), have shown that administration of androgens to the human female or of oestrogens to the male does not alter the incidence. In addition, as Davidson and Flute (1962) observe that in some cases of human intersex, drumsticks may be found in spite of the presence of testicular tissue, and, as Davidson and Smith (1963) also point out, the ultimate proof that drumsticks are not a hormone dependent characteristic is found in the natural and artificial chimaeras, where female cells arising from naturally or transplanted marrow, persist in the male blood stream.

Another possible objection to tissue sex chromatin and drumsticks being a similar manifestation is the difference in size between the two structures, the average diameter of sex chromatin being 1μ, and of drumsticks, 1.5μ. Davidson and Winn (1961) however, have shown that this difference depends upon the method of preparation, for when neutrophils are examined in tissue

sections, drumsticks and tissue sex chromatin have the same average dimensions.

Although it is now generally agreed that drumsticks bear some relation to sex chromatin, Ashley (1957) and Pfeifer (1962) maintain that drumsticks and sex chromatin are two different entities. In support of his argument, Ashley claims to have observed a heterochromatic mass resembling tissue sex chromatin in polymorphonuclear leucocytes which also contained drumsticks and concludes that "the satellite body of Davidson and Smith is not identified with the heterochromatic mass and must be regarded as a sex characteristic". Ashley's observation of this phenomenon, however, has not been confirmed by other workers.

There has been some disagreement about the nature of these masses of chromatin which various workers have described within the nuclei of certain cells in the white cell series. For example, whereas Ashley (1957) claims to have observed chromatin masses resembling tissue sex chromatin in all the cells of the myeloid series in bone marrow preparations in the female and Murthy and von Haam (1962) have described sex chromatin in late myelocytes, segmented and unsegmented polymorphs and monocytes, Davidson and Smith (1963) maintain that these chromatin masses

in marrow cells and in monocytes may be present in either sex. Riis (1958) has also described a chromatin mass resembling sex chromatin, in lymphocytes, but Davidson and Smith (1963) note that since the technique used was completely different from those normally used to demonstrate either sex chromatin or drumsticks, his observations must be regarded with caution.

It is now thought that the drumstick is merely an extruded sex chromatin nodule (Murthy and von Haam 1962), and that the sessile nodule is the early form of the drumstick (Davidson and Smith 1963), but the reason for the protrusion of the drumstick is obscure. It has been suggested by Davidson and Winn (1961) that the production of the drumstick might indicate a rejection phenomenon from the active part of the nucleus when not involved in cell division. Alternatively, Davidson and Smith (1963) suggest that it is possible that this mechanism is to bring the chromatin into closer relationship with the cytoplasmic elements. If, however, drumsticks and sex chromatin are similar manifestations, then the proposal that sex chromatin is largely inactive would tend to favour the former of the two theories.

I have now briefly considered the historical background and normal appearance of both the human chromosomes and sex chromatin, together with methods for detecting the latter in man. I now

turn to the applications of these latter techniques and some of the associated chromosomal findings.

PART 3

APPLICATIONS OF NUCLEAR SEXING

Applications of Nuclear Sexing

The technique of nuclear sexing has been put to use in several fields including the ante-natal sexing of foetuses, as previously described, and in the detection of sex for medico-legal purposes by examining tissues such as skin, or, according to Davidson and Flute (1962), bloodstains. It has also been shown that the technique is of value in the determination of the primary sex ratio, by obtaining information about the differences in mortality between the sexes during gestation, and Hienz and Stoll (1962), who are workers in this field, have shown that during gestation, there is an increased incidence in the mortality of male embryos which reaches its peak during the 3rd and 4th months of pregnancy. In addition, nuclear sexing has been used, for example, by Woodruff and Lennox (1959), in the study of the fate of grafts, and has also assisted in determining the origin of certain placental tissues such as the septa placenta, which have been shown by this technique to be of maternal origin (Austin 1962).

An extremely interesting application of nuclear sexing has been the study of sex chromatin in tumour cells, and reviews on this subject by Ashley (1962), Tavares (1962)

and Lennox (1963a) cite many workers in this field. In general, as Tavares states, apart from the teratomata, "benign and malignant neoplasms show a nuclear sex which is identical to that of the bearer". Lennox notes that sex chromatin is rarely seen in malignant tumours in males, but is seen with low frequency in malignant tumours in females, because in rapidly growing tumours many damaged or dying cells are undergoing karyolysis or pyknosis. In addition, there is less chance of detecting sex chromatin in large tumour nuclei such as are found in malignant lesions.

Nuclear sexing has been useful in confirming the origin of certain tumours. For example, examination of chorio-carcinomas by Park (1957b) has supported the foetal origin of this tumour. On the other hand, with regard to the interesting group of teratomatous tumours, various theories have been advanced. For example, in his textbook on the Pathology of Tumours (1960), Willis says that "teratomas are tumours arising from foci of plastic pluripotential embryonic tissue which escaped from the influence of the primary organiser during early embryonic development, this escape being in some way related to disturbances emanating from the invaginated organising tissues of the primitive streak and so

affecting median or paramedian parts in close relationship to these tissues. The affected primordia as it grows, differentiates in accordance with its own intrinsic 'labile determination', producing a variety of tissues foreign to the part in which it grows".

However, the application of nuclear sexing to teratomas by workers such as Hunter and Lennox (1954), Myers (1959) and Theiss et al. (1960) has raised the possibility of another form of aetiology. These investigations have shown that whereas, as expected, the teratomas in females are chromatin-positive, those arising in males may be chromatin-positive or chromatin-negative, or even, as Myers' detailed examinations have demonstrated, may present a mingled mosaic pattern of chromatin-positive and chromatin-negative tissues. In order to explain this phenomenon, Hunter and Lennox, in 1954, originally suggested that of all the theories of aetiology, "the simplest would seem to be that teratomata derive from the fusion of two gametes (or at least of haploid cells) which would always produce an XX zygote in the female, but in the male, might in theory produce XX, XY or even YY."

If this theory were correct, the YY cell being

presumably non-viable, the proportion of chromatin-negative (XY) to chromatin-positive (XX) tumours in the male should be 2 : 1. However, most of the figures published by various workers listed by Lennox (1963a) and Ashley (1962) show a sex ratio of 1 : 1 which is more in favour of the theory that teratomas arise by single chromosomal re-duplication in a haploid cell. As pointed out above, Myers (1959) demonstrated that some teratomas containing sex chromatin might have a mosaic pattern. Lennox (1963a) observed that, previously, any such tumour containing sex chromatin was regarded as being chromatin-positive. Being aware of this possibility of mixed sex chromatin pattern, Theiss and his colleagues (1960) investigated 96 teratomas, and, in addition to mosaic appearances in 3, found that of the remainder, the ratio of chromatin-positive to chromatin-negative tumours was 2 : 1. This finding tends to support the parthenogenetic theory. Although Myers thought that "the occurrence of mosaic tumours appears to rule out parthenogenesis as an explanation of 'female' teratomata in males", Lennox (1963a) proposed, in order to overcome this difficulty, that teratomas may arise from a group of abnormal cells rather than from one cell alone. However, the question of parthenogenesis

versus reduplication does not yet appear to be finally settled.

With regard to teratomas of extra-gonadal origin, Willis' or Ashley's suggestion that they are "derived from germ cells which have become misplaced during ontogeny" appears as satisfactory as any.

Although nuclear sexing in these various fields has yielded interesting and useful results, the main application of this technique has been in the large complicated group of anomalies of sex development, or the intersexes, in which nuclear sexing, chromosome investigation and biochemical studies of defects of hormone synthesis have aided in the understanding of some of the intersex conditions.

The term "intersex", according to Lennox (1960), includes any kind of deviation "social, psychological, anatomical, endocrine or chromosomal, from the strict norms of male and female". Intersexes have long been a subject of interest because of the ambiguity, in some cases, of the external genitalia. Indeed the "hermaphrodite" group owes its name to Greek mythology, the term being derived from "Hermaphroditos" a minor Greek deity said to be the child of Hermes and Aphrodite, and frequently represented in classical sculpture

(according to Ashley 1962), as "a composite being usually with well formed breasts and an equally well formed masculine phallus". Ashley continues that "the Romans added the concept of functional bisexuality to the anatomical mixture seen in Hermaphroditos".

There have been many systems of classification suggested for the complicated intersex group. Before nuclear sexing techniques were introduced, the histological appearance of the gonads was taken as the ultimate criterion of the sex, but nuclear sexing and chromosomal studies have necessitated a more detailed classification together with the inclusion of further large groups of intersexes such as Klinefelter's and Turner's syndromes.

In his review of intersex, Ashley describes several different systems of classification adopted by various workers, and shows how these vary according to the author's concept of the aetiology, for example, embryogenetic, chromosomal, etc. Further classifications have been introduced, e.g. those described by Polani (1962), but the classification proposed by Ashley, based on nuclear sex together with gonadal and somatic sex findings is simple and fairly comprehensive.

Ashley divides anomalous sex development into three

broad groups. In his first group, the nuclear, gonadal and somatic sex are in agreement. This group includes cases of hypogonadism due to genetic or environmental causes leading to infertility and possible failure of secondary sex characteristics, together with psychosexual anomalies such as homosexuality and transvestism. The second group consists of cases in which the nuclear and gonadal sex are in agreement but in which somatic sex anomalies are present. This group includes the pseudohermaphrodites. In the third group, the nuclear and gonadal sex are not in agreement, and Ashley includes in this group true hermaphrodites, together with Turner's syndrome, Klinefelter's syndrome and other chromosomal anomalies.

Nuclear sexing, combined with the clinical findings is of special value, particularly in the pseudohermaphrodite group, in determining in which sex a child born with ambiguous external genitalia should be reared. Plastic surgery can often be performed where necessary. Later in life, especially as Overzier (1963a) observes "the decision will frequently go against the nuclear sex which is primarily of importance only in order to make a diagnosis".

The pseudohermaphrodites are of interest in that

much of the aetiology has been elucidated, in the case of some of the female pseudohermaphrodites by the investigation of the biochemical synthesis of hormones, and in the case of some of the male pseudohermaphrodites by castration experiments in animals.

Female pseudohermaphroditism (Masculinisation of the female)

The most common of the female pseudohermaphrodite group of intersexes is the well known condition of congenital adrenal virilism or hyperplasia. This disease, details of which are amply given by Bierich in his review (1963) usually presents in infancy or early childhood when it is associated with hyperplasia of the adrenal cortex. The syndrome can also occur in older subjects in whom it may be due either to a form of congenital adrenal hyperplasia manifesting itself later in life, or to an adrenal cortical tumour which usually is malignant (Bierich 1963).

The indicence of congenital adrenal hyperplasia has been estimated at 1 in 50,000 (Polani 1962). It is familial and is inherited by an autosomal recessive gene

(Childs et al. 1956). The nuclear sex is chromatin-positive (Moore et al. 1953). The clinical manifestations are very variable, the most characteristic feature being virilisation of the lower female genital tract and the external genitalia. This virilisation may occur alone, or may rarely be accompanied by hypertension, or, in a quarter of cases, according to Bierich (1963), it may be accompanied by an Addisonian-like electrolytic defect with loss of sodium and chloride ions. In this latter variant of the disease, the course may be very rapid with death in the first few months of life due to adrenal failure. Nuclear sexing is therefore of great practical importance in the vital early diagnosis of this condition which is eminently treatable with cortisone or other suitable steroids. On the other hand, the hypertensive form of the disease may not become fully manifest until after puberty, or again, the patient may live for years showing only the features of virilism (Ashley 1962). Congenital adrenal hyperplasia may also occur in the male, in whom the salt losing form of the syndrome, according to Ashley (1962) may be the only one readily recognisable early in life.

In the urine, the level of 17-ketosteroids is raised and abnormal steroids are present, whereas in the blood, the level of adrenocorticotrophic hormone (A.C.T.H.) is increased. It has been shown by Jailer and his colleagues (1955) and by Bongiovanni and Eberlein (1958) that the initial defect in congenital adrenal hyperplasia is a blockage of some of the enzyme systems, the clinical manifestations depending on the enzymes blocked. The consequent inadequate formation of steroids is thought to lead to a 'negative feed back' mechanism in the pituitary, producing an increased secretion of A.C.T.H., which in addition to causing the adrenal hyperplasia, adds to the severity of the anomaly and to the formation of other steroids such as androgens.

Apart from this important clinical condition, female pseudohermaphroditism may also be caused by steroid hormones which are exogenous to the foetus. For instance, Polani (1962) cites work done by Wilkins (1960) who has found that certain steroid hormones such as testosterone, progesterone or diethyl-stilboestrol given to the mother for the prevention of habitual abortion may produce female pseudohermaphroditism in the child, although in some of the cases given progesterone

and diethyl-stilboestrol, and in other cases of masculinisation where the foetus had not been exposed to external steroids, Wilkins, (according to Polani), has postulated that a defect of maternal steroid metabolism might exist. Polani also mentions that masculinisation of a foetus may occur when the mother has a functioning arrhenoblastoma.

According to Lennox (1960) female pseudohermaphroditism which is not due to hormonal masculinisation is very rare, most cases comprising complex congenital malformations involving the urinary and genital tracts.

Male pseudohermaphroditism

Feminisation of the male, or male pseudohermaphroditism is more frequently encountered than female pseudohermaphroditism. Detailed clinical descriptions are given by Ashley (1962) and Overzier (1963a) who show how the degree of feminisation of the external genitalia may vary considerably, ranging from the commoner appearance of predominantly male to the less common form of predominantly female. Although testicular tissue is present in these subjects, structures of Müllerian duct origin, i.e. a rudimentary

uterus, vagina and Fallopian tubes may also be found. The testes are poorly developed and vary in position, being found either in the scrotum or in the position of the ovaries, or in an intermediate position showing some degree of descent. The histology is usually abnormal and the seminiferous tubules are defective (Grumbach and Barr 1958). The nuclear sex is chromatin-negative.

It is thought that the aetiology of male pseudo-hermaphroditism lies in some physical deficiency, the evocator normally produced by the embryonal testis failing to produce full masculinisation of the reproductive system. Whereas, in the embryo, the differentiation of the gonads depends on whether the germ cells reaching the gonadal site have the sex chromosome complex XX or XY (Lennox 1960), the differentiation of the genitalia appears to depend on different factors.

In the embryo, both Müllerian and Wolffian ducts are present, the Müllerian ducts capable of differentiating into Fallopian tubes, uterus and upper vagina, and the Wolffian duct capable of forming the epididymis, vas deferens, and seminal vesicle.

Classical experiments by Jost (1947, 1953), have

shown that the differentiation of the genitalia depends ultimately on whether or not a male gonad is present. Jost has demonstrated that when female rabbit foetuses are castrated at any stage of development, the Müllerian duct persists, the Wolffian duct atrophies, and the rabbits develop as females. With male rabbit foetuses, however, castration leads to varying results. If these male foetuses are castrated at an early stage of development, the Müllerian duct persists and they develop as females, whereas, if they are castrated after a certain critical period of time, the Wolffian duct persists and the rabbits develop as males. These observations indicate that whereas ovaries are not necessary for female development, the positive action of an evocator from the embryonal testis is essential during a certain critical period for normal male development to occur. This masculinising evocator also appears to have a local action on tissue, for Jost has also shown that a unilateral graft of embryonal testis into a female embryo stimulates the Wolffian duct and suppresses the Müllerian duct at the site of the transplant.

According to Lennox (1960), then, it is highly

probable that "the ordinary male pseudohermaphrodite results from a partial failure of the endocrine activity of the testis during the crucial stage of genital differentiation: the many anatomical variants reflect differences in the degree and timing of the failure".

There remains the extremely rare condition of congenital lipid hyperplasia of the adrenal which may cause feminisation in the male. This condition was described by Prader and Siebenmann (1958) in 6 babies of female phenotype, in 3 of whom the internal genitalia were female, testicular tissue being present in the remaining 3 babies. All the cases had signs and symptoms of adrenal failure resembling Addison's disease, and all died within the first year of life. At autopsy, the adrenals were yellow and enlarged, showing diffuse and nodular hyperplasia, the cortical cells containing large amounts of lipid and cholesterol. The aetiology of this rare condition was suggested to be an enzymatic block in one of the first steps of steroid synthesis with failure of conversion of cholesterol into the precursors of adrenal and testicular steroids, consequently leading to Addisonian crises and feminisation of the male babies.

Testicular feminisation

Testicular feminisation has been given the term of "complete external male pseudohermaphroditism" by Ashley, who, in common with other workers, e.g. Grumbach and Barr (1958), has classified this syndrome as an extreme form of male pseudohermaphroditism. However, other authors, such as Polani (1962), have separated testicular feminisation from this latter intersex group on the grounds that the external genitalia are not usually intersexual, the clinical picture is consistent and unequivocal, and the inheritance which is striking, is different from other forms of intersexuality. This syndrome is one of the most inconspicuous forms of intersexuality, and because the nuclear sex is at complete variance with the phenotypic sex, it has, on occasion, been placed by other workers, e.g. Lennox (1960), into a separate group of so-called "sex-reversals" along with Klinefelter's syndrome and Turner's syndrome. Whereas in the latter two syndromes, however, there is an abnormality in the sex chromosome constitutions, subjects with testicular feminisation have been shown to have the normal XY chromosome constitution found in the male corresponding with the

chromatin-negative nuclear sex. (Jacobs et al. 1959a).

According to Hauser (1963a), de Quervain was actually the first to write about testicular feminisation in his textbook of Surgery in 1923, when he said "in spite of plaits and a girl's name, the structure in the inguinal canal may be a testicle". The term "testicular feminisation" was first applied by Morris (1953), who collected numerous cases from the literature and added two of his own. Following Morris' work and description of the syndrome, there have been many other publications about this interesting condition.

Clinically, the patient, of female phenotype, may present either in childhood with an inguinal hernia in which testicular tissue is found at operation, or may seek advice in adult life for amenorrhoea or sterility (Lennox, 1960).

On external appearance, although the stature is usually normal (Ashley 1962), the patient may, on the other hand, be tall and eunuchoid (Morris 1953). Secondary sex characteristics are female, and although the nipples are occasionally infantile (Morris 1953), the breasts may be normal or even overdeveloped. The external genitalia are

female in appearance but pubic and axillary hair may be absent or scanty. According to Grumbach and Barr (1958), occasional subjects are less markedly feminised, and show more masculine external genitalia together with lack of breast development. The vagina which may be 'blind' is either normal or short in length, (Polani, 1962), and although the uterus and Müllerian derivatives are nearly always absent or rudimentary in form, Ashley cites occasional cases in which menstruation has occurred.

The gonads, consisting of the testis, epididymis and proximal parts of the vasa deferentia, may be intra-abdominal, or present within the inguinal canal or labia majora. When the testes are undescended, there is a tendency for malignant change to occur (Morris 1953).

The histology of the testes in this condition, is described by Hauser (1963a) in his review. The seminiferous tubules which are immature or maldeveloped are narrow and often have no lumen. Although, according to Grumbach and Barr (1958) and other workers cited by Hauser, the tubules are lined largely by Sertoli cells, Hauser states that in his material, Sertoli cells are few, the majority of the cells being undifferentiated. Spermatogonia may be occasionally

seen and Hauser cites several workers who have identified spermatocytes and even spermatozoa. The interstitial cells of Leydig are well developed and may be increased in number. Tubular adenomata frequently occur, (Morris, 1953).

According to Polani (1962), oestrogens and androgens are secreted at normal levels for women, and after castration, menopausal symptoms often develop.

With regard to the aetiology, the exact mechanism of the defect is unknown. Although it was previously postulated that the syndrome was associated with an XXY sex chromosomal abnormality (Danon and Sachs 1957), it has been shown that the chromatin-negative nuclear sex corresponds with a normal XY male sex chromosome pattern (Jacobs et al., 1959a).

The syndrome is probably, however, a sex-linked anomaly, for testicular feminisation is familial and is transmitted through the maternal line to affect only males, but, as Polani (1962) observes, since the affected males are sterile, it cannot readily be determined whether the condition is transmitted as a sex-linked recessive character or as a sex-linked autosomal dominant defect.

Various suggestions have been made regarding the nature of the hormonal anomaly in this syndrome. For example, Lennox (1960) states that "the facts strongly suggest a simple maturation error in (presumably) the Leydig cells of the testis, leading to the production of the wrong hormones both in utero and at puberty". In view of the unusual tissue response, it has been suggested by Morris (1953) that the oestrogen secretion may be different from normal and Polani (1962) mentions that it has also been postulated that the syndrome is a result of target-organ resistance.

True Hermaphroditism

This interesting form of intersex has been placed by Ashley into the group in which the nuclear and gonadal sex are not in agreement.

Detailed reviews of this intersex group have been written by workers such as Ashley and Overzier (1963c) who cite numerous cases from the literature and describe the various clinical and anatomical variants which may occur. Ovarian and testicular tissue are present in various combinations, so that for example, true hermaphrodites may have

one gonad forming a testis and the other gonad forming an ovary. More commonly, one or both gonads consist of an 'ovotestis' containing both ovarian and testicular tissue. According to Overzier (1963c), the ovary is usually found in the normal position or slightly lower, but whereas the testis is usually present in the scrotum, both the testis and ovotestis may be found anywhere along the normal path of descent of the testis. The internal genitalia usually correspond to the gonadal or dominant gonadal tissue on that side of the body, so that in most cases, a tube leads to the ovary and a vas deferens to the testis. True hermaphrodites usually have a uterus, vagina or urogenital sinus, and, in most cases, a prostate (Overzier, 1963c). The external genitalia show varying degrees of anomaly, but 'pragmatic males predominate' (Lennox, 1960).

The histological appearance of the gonads is described by Overzier. The testes may show any degree of maturation and development including complete spermatogenesis. The lining of the tubules which consists mainly of Sertoli cells, and occasionally of spermatogonia and spermatocytes, atrophies. The interstitial cells of Leydig increase in number.

The ovaries may sometimes appear normal (Ashley, 1962), but although in two-thirds of cases of true hermaphroditism menstruation occurs, no known true hermaphrodite has given birth to a child (Overzier 1963c).

With regard to the ovotestis, the testicular component is usually separated by a fibrous band from the ovarian tissue, but sometimes, mingling of the two types of tissue may occur. According to Ashley, the testicular component which is usually larger than the ovarian element, shows the histological features of an undescended testis, whereas the ovarian tissue presents a more normal appearance. The nuclear sex of these patients may be chromatin-negative, or more usually, according to Grumbach and Barr (1958), chromatin-positive.

Chromosome investigations have been applied to a few true hermaphrodites, and the results have been reviewed by Polani (1962). In the majority of cases examined, the sex chromosome pattern has been reported as XX, with occasional minor chromosomal irregularities. A few cases, e.g. those reported by Sandberg et al (1960), have the XY sex chromosome complex.

A possible mosaic pattern of XX/XXX has been

described in one case by Ferguson-Smith and his colleagues (1960b), and an XY/XO complex has been reported by Hirschhorn et al (1960) in an intersex patient in whom biopsy of one gonad showed "testicular and ovarian tissue in an immature state". Further examples of the XY/XO complex (with occasional minor modifications) have been reported in other intersex patients, in some of whom the gonads have consisted of a testis and an ovarian 'streak' similar to that found in the intersex condition of ovarian dysgenesis. Cases of this type present some difficulty in classification which will be discussed in the following section dealing with Turner's syndrome.

Turner's Syndrome

Turner's Syndrome

Turner's syndrome, which, as previously mentioned, has been referred to by some authors as one of the so-called 'sex reversals', has been included by Ashley, in the category of intersex in which the nuclear and gonadal sex are not congruous. The syndrome is found in phenotypic females with ovarian tissue, but in whom, in the majority of cases, the nuclear sex is chromatin-negative.

The syndrome owes its name to Turner, although,

according to Hauser (1963b), various features of the syndrome had been previously described by several workers, including Ullrich who, in 1930, reported some of the pre-puberal features. In 1938, Turner described a group of anomalies occurring in females, consisting of neck webbing, cubitus valgus, short stature and sexual infantilism. He suggested that the primary defect lay in the pituitary, but in 1942, Albright and his colleagues demonstrated that because of the excess of pituitary gonadotrophins in the urine, the defect lay instead with the gonads which in fact were shown to consist of 'streaks' of gonadal tissue containing no follicular or germ cell activity (Wilkins and Fleischmann 1944).

As the original concept of the syndrome has been broadened, it has been shown that the clinical features are many and variable, with the result that varied nomenclature has been used to describe the condition, the differing terminology, as Polani (1962) has observed, giving rise to much confusion. Of the numerous eponyms listed by Hauser (1963b), those most frequently in use are 'Turner's syndrome' and 'gonadal' or 'ovarian dysgenesis'. Some workers, however, e.g. Hauser, have preferred to reserve the term

'Turner's syndrome' for the clinical features originally described by Turner.

As previously mentioned, the clinical features of ovarian dysgenesis (to adopt the nomenclature suggested by Polani 1962), may vary considerably, and numerous anomalies have been described, details of which are given by Polani (1961a), Ashley (1962) and Hauser (1963b). As originally reported by Turner, sexual infantilism is present; the breasts are usually underdeveloped, and the nipples, which are very small with almost completely unpigmented areolae, are widely separated. Axillary and pubic hair is scanty. Apart from the ovaries, the internal genitalia are completely developed but are immature. The tubes are narrow and apparently long, the uterus infantile, the endometrium scanty or absent, and the vagina small. Primary amenorrhoea is nearly always found, although, according to Ashley (1962), a few cases have been reported in which menstruation has occurred. The gonads consist of whitish streaks of fibrous connective tissue in which germ cells and follicular activity are absent. According to Ashley, however, a few cases have been described in which ovarian follicles were present, and in one instance a

chromatin-negative phenotypic female has proved to be fertile, (Bahner et al, 1960).

Apart from these anomalies of secondary sex characteristics and gonads, numerous other abnormalities may be present. The birth weight tends to be low and the stature, which is small, is not usually more than 4 ft. 9 ins. (Polani 1961a). The trunk is broad and may show the 'shield chest' deformity. Neck webbing may be present and this feature has been used by Polani (1962) to divide subjects with ovarian dysgenesis into two large groups consisting of those with, and those without neck webbing. He states that among those with neck webbing the incidence of chromatin-negative nuclear sex together with various associated malformations is greater. These malformations include coarctation of the aorta and other forms of congenital heart disease, renal anomalies, and various skeletal anomalies involving especially the face, vertebral column, pelvis, limbs and knees. Slight intellectual subnormality is also more frequently encountered in this group, but is not a prominent feature of the syndrome.

Other features which may be present include delayed ossification and generalised osteoporosis, webbing of the

fingers or axillae, low-set enlarged ears, mild ocular defects and congenital lymphoedema of the limbs, feet and hands which is found in one quarter of all cases, (Polani 1961a). Various other abnormalities which may be found are amply described by Hauser (1963b).

Although the majority of patients have this type of clinical picture, a few remaining subjects with ovarian dysgenesis have been placed by various workers into separate groups because of their different clinical features. For example, some patients have the feature of gonadal dysgenesis alone, and because of the absence of the other congenital abnormalities, this variant has been termed 'pure gonadal dysgenesis'. (Harnden and Stewart, 1959). In other subjects with ovarian dysgenesis in whom the clitoris is enlarged, the gonadal streaks may contain large numbers of hilar cells (Jones et al 1963) which have been described by Grumbach and Barr (1958) as resembling Leydig cells. This group of cases is termed by some authors "gonadal dysgenesis with phallic enlargement", (Grumbach and Barr 1958, Polani 1962). The third syndrome which has been reported by some workers as a variant of ovarian dysgenesis is termed "gonadal dysgenesis with male

pseudohermaphroditism" (Grumbach and Barr 1958, Polani 1962). Subjects in this interesting group have been described as having the appearance of a male pseudohermaphrodite with ambiguous external genitalia. According to Polani (1962) one gonad is composed of testicular tissue and the other is represented by a 'gonadal streak' similar to that found in the usual form of ovarian dysgenesis.

Difficulty is obviously present in the classification of this group of patients for they may be said to present features of ovarian dysgenesis, male pseudohermaphroditism and true hermaphroditism. Several patients with various features of these intersex groups have been described, and in a recent review of the situation, Sohval (1963) has proposed the term of 'mixed gonadal dysgenesis'. This category, according to Sohval applies to subjects of male and female phenotype who have an enlarged clitoris or phallus, together with a vagina, uterus and Fallopian tubes. The gonads may consist of intra-abdominal testes, or of a testis together with a gonadal streak. These subjects are chromatin-negative. The difficulty, emphasised by Sohval, in classifying these patients, serves

to reinforce Ashley's statement that various anomalies of intersex cannot be placed "neatly in a pigeon-hole with the tacit assumption that there are clearly marked differences between the different anomalies of development and the different symptom complexes".

Turner's syndrome has been described in the male as well as in the female. These male subjects, (according to Ashley), may have webbing of the neck, shortness of stature, peripheral oedema, coarctation of the aorta and various skeletal anomalies. Hypospadias may be present, and the testes may show abnormalities of structure or function, e.g. unilateral or bilateral maldescent. Post-pubertally, according to Polani (1962), the evidence of testis involvement is shown by a reduction in size with absent or impaired spermatogenesis. The few cases of Turner's syndrome in the male whose chromosomal pattern has been studied have been found by Court Brown and his colleagues (1960) and other workers cited by Polani (1962) to be chromatin-negative with a normal chromosome number of 46 and a male XY sex chromosome complex. According to Ashley "the genetic anomaly of Turner's syndrome in the male, is, apparently as yet

beyond our analytical capacities. There may be some qualitative change in the chromosomes or possibly a minor translocation too small to be detected. This remains one of the problems still to be solved".

However, in females with ovarian dysgenesis, the application of nuclear sexing techniques together with chromosome analysis has revealed some extremely interesting anomalies.

In 1954, Polani and his colleagues applied nuclear sexing techniques to three patients with ovarian dysgenesis and found that they were chromatin-negative. Subsequent investigations by workers such as Segal and Nelson (1957) have shown that although the majority of patients with ovarian dysgenesis are chromatin-negative, some may be chromatin-positive. Results of colour blindness studies in chromatin-negative subjects suggested that only one X chromosome was present, (Polani et al, 1956), and chromosome investigations later showed that this indeed was the case, for in the majority of chromatin-negative cases, only 45 instead of 46 chromosomes are present, and the sex chromosome pattern is XO (Ford et al, 1959b).

However, further investigations of both chromatin-

negative and chromatin-positive subjects have shown that other sex chromosome patterns may exist including both normal and abnormal complexes. Normal sex chromosomes and sex chromatin patterns have been found in the variant 'pure gonadal dysgenesis'. Some of these patients are chromatin-negative and have the sex chromosome complex XY (Harnden and Stewart 1959). According to Polani (1962) there is slight evidence that the condition, in some cases, may be genetically determined and may be related to a point, or single gene mutation.

In other subjects with pure gonadal dysgenesis, the nuclear sex is chromatin-positive and the sex chromosome pattern is XX (Jacobs et al 1961). It has been suggested by Jones and his colleagues (1963) that in these cases, a mechanical or viral factor may have interfered with the normal development of germ cells or their migration to the ovaries.

With regard to the abnormal sex chromosome complexes, numerous abnormalities in addition to the XO pattern have been described. For example, in chromatin-negative subjects with ovarian dysgenesis, various mosaic

complexes have been reported including XO/XYY (Jacobs et al 1961), XO/XX (Sandberg et al 1960), and XO/XY/XYY (Jones et al 1963). The XO/XY mosaic complex has been described both in the usual form of chromatin-negative ovarian dysgenesis (Jacobs et al 1961) and in the variant of mixed gonadal dysgenesis, in which minor modifications of this mosaic complex may also exist and are described by Sohval (1963).

Abnormal mosaic complexes have also been described in chromatin-positive cases of ovarian dysgenesis e.g. XO/XX, XO/XXX (Jacobs et al 1961), XO/XX/XXX (Hayward and Cameron 1961, Jones et al 1963). An interesting feature in some of the subjects in whom three X chromosomes form one of the cell lines, is the presence, in some of the tissue nuclei, of two sex chromatin bodies instead of one. This feature has also been described in other sex chromosome anomalies including the 'triple-X syndrome', and some variants of chromatin-positive Klinefelter's syndrome. The significance of this will be discussed in part 6.

Several other extremely interesting anomalies have been described in chromatin-positive patients with

ovarian dysgenesis. For example, morphological abnormalities of the X chromosme including deletions and the formation of isochromosomes have been reported, (Fraccaro et al 1960a, Jacobs et al, 1960, 1961), and in addition, Grumbach and Morishima (1962) have demonstrated the presence of sex chromatin in XO cells of a patient with ovarian dysgenesis. These abnormalities will be considered in greater detail in part 6, when discussing the nature of sex chromatin and its relationship to the X chromosome.

With regard to the application of nuclear sexing to the intersexes, I have discussed 'hermaphroditism,' together with testicular feminisation and Turner's syndrome. There remains the condition of Klinefelter's syndrome which, as I have previously noted, has been classified by Ashley in the third of his intersex groups in which the nuclear and gonadal sex are not congruous. Klinefelter's syndrome, together with its variants, will be described in the following part of this thesis.

PART 4

CHROMATIN - POSITIVE KLINEFELTER'S SYNDROME

Chromatin-Positive Klinefelter's Syndrome

The term 'Klinefelter's syndrome' is derived from a condition described in 1942 by Klinefelter and his colleagues. The title of their paper summarised the main features as those of a "syndrome characterised by gynaecomastia, aspermatogenesis, without a-Leydigism, and increased excretion of follicle stimulating hormone". The testes were described as being small, showing histologically hyalinisation of all the tubular elements, but with intact Leydig cells. The secondary sex characteristics were defective and the level of 17-ketosteroid hormone excretion in the urine varied from normal to markedly decreased.

This original concept was expanded by Heller and Nelson, who, in 1945, reviewed 20 patients with the syndrome. They demonstrated that, whereas small testes, azoospermia, and elevation of the level of gonadotrophin excretion in the urine were constant features, the gynaecomastia, mentioned by Klinefelter, together with other features such as eunuchoidal skeletal proportions, high pitched voice, poor muscle development, scanty pubic and facial hair, and underdeveloped external genitalia, although present in some patients, were

variable features, and were not considered by Heller and Nelson to be essential to the syndrome.

Over the following eleven years, little was contributed to the original concept of the syndrome, and there was a tendency to classify all patients with small testes and azoospermia as being cases of 'Klinefelter's syndrome'. In 1956, Plunkett and Barr, and Bradbury and his colleagues, together with other workers cited by Overzier (1963b) in his review, made a major contribution towards the understanding of this syndrome, when, by applying the technique of nuclear sexing to patients with Klinefelter's syndrome, they found that some of these phenotypic males possessed chromatin-positive nuclei. Speculation regarding the nature of Klinefelter's syndrome then increased. Several surveys of patients with Klinefelter's syndrome were rapidly carried out in order to investigate the nuclear sex, with some varied results, for although, for example, Lennox and his colleagues (1958) found that in their material more cases were chromatin-negative than chromatin-positive, other workers, e.g. Nelson (1956), and Segal and Nelson (1957), have reported that in their surveys the majority, (about 80%), of patients were chromatin-positive.

For convenience, therefore, Klinefelter's syndrome can

be divided into chromatin-positive and chromatin-negative forms. The former, chromatin-positive Klinefelter's syndrome, which presents abnormalities of sex chromatin and sex chromosomes, is the form which will be largely discussed in this chapter. The latter, chromatin-negative Klinefelter's syndrome, shows no anomalies of sex chromatin or sex chromosomes. Its aetiology is different from the chromatin-positive form, but because, clinically, it presents some similar features, it will be briefly compared with chromatin-positive Klinefelter's syndrome after the clinical and histological features of the latter have been described.

Attempts have been made to find a more suitable name than 'Klinefelter's syndrome'. When nuclear sexing techniques were first applied, Nelson (1956) introduced the terms 'true' and 'false' Klinefelter's syndrome to represent respectively the chromatin-positive and chromatin-negative forms, but the latter terms are much more accurate and suitable. Further names have been suggested for the syndrome such as 'testicular dysgenesis' by Plunkett and Barr (1956), 'pseudomales' by Witschi and his colleagues (1957), 'seminiferous tubule dysgenesis' by Grumbach et al. (1957), 'hyperplastic medullary gonadal dysgenesis' by Stewart (1958) and finally 'primary

micro-orchidism' by Ferguson-Smith in 1958. However, although the term 'Klinefelter's syndrome' is not entirely suitable, it has the advantage of being recognised as a clinical entity, is widely known, and is convenient.

Clinical features of chromatin-positive Klinefelter's Syndrome

Many of the clinical features were described in the earlier papers on Klinefelter's syndrome, and these, together with later findings are adequately detailed in recent reviews (e.g., Ashley 1962, Overzier, 1963b).

In younger age groups there may be no clinical signs or symptoms, and the syndrome usually manifests itself during adolescence when there may be delay of puberty together with the development of gynaecomastia and other features. The adult, on the other hand, usually presents with infertility, or sometimes gynaecomastia. The patient may be tall and have a eunuchoid stature with delay in fusion of the epiphyses of the long bones leading to a tendency to excessive growth in the length of the extremities. Stewart and his colleagues (1959) emphasised that in some cases the arm span is greater than the height and the lower segment of the body (pubis to sole) greater than the upper segment (pubis to crown). The pitch

of voice may be high, and hair on the face, axilla and pubis may be sparse in amount. There may be variation in muscle development and strength. The hair at the temples may show lack of recession. The external genitalia show reduction in size of the testes, and sometimes, also, the penis. The interstitial cells of Leydig in the testis tend to fail earlier than normal and the so-called male climacteric begins sooner. Osteoporosis may occur.

As far as hormone estimations are concerned, according to Ashley (1962), the increased secretion of follicle stimulating hormone, has been confirmed in numerous cases; Overzier (1963b), however, mentions that several exceptions are known. Oestrogen levels are not increased and the excretion of 17-ketosteroids in the urine just reaches the lower limits of normal (Overzier, 1963b).

Heller and Nelson (1945) have emphasised that the majority of the clinical signs just described, need not be present in these patients, and Ashley (1962) has gone so far as to say that "the only constant feature so far observed in this syndrome is infertility". However, as Ashley points out, the diagnosis of a fertile case of Klinefelter's syndrome "must arise largely by chance, and, of course, rigid proof of paternity

would be necessary in view of the unusual nature of the phenomenon". According to Lennox (1963b), many workers have looked for such cases, and two of the most recent claims of fertility have been made by Kaplan et al. (1963) and Warburg (1963). In Kaplan's case, at least, fertility has not been proved conclusively, but in the case described by Warburg, blood group studies do not contraindicate the possibility.

Histological features

The normal male breast consists of a moderately dense fibrous connective tissue stroma containing a few ducts, but when gynaecomastia is present, there are certain histological changes which are described in detail by Karsner (1946). According to Karsner, in the breast, there is proliferation of connective tissue which is dense in the general stroma and loosely arranged in the periductal areas. In addition, there is a periductal or more widespread infiltrate of lymphocytes, plasma cells and large mononuclear cells together with occasional eosinophils and polymorphs. The ducts show branching, elongation or multiplication, and often contain secretion which may be discharged but rarely consists of true colostrum or milk. Mitotic activity may sometimes be present in the

epithelium which may show much hyperplasia, sometimes of a papillary pattern, but with rare formation of true intraductal papillomas.

Although Karsner states that true acini are never found, Sandison (1962) has noted apparent acinar formation with well formed lobules in the breasts of two chromatin-positive Klinefelter cases from the material of this department.

The histological features of the testis in chromatin-positive Klinefelter's syndrome present many interesting features. The normal adult testis (Fig. 5) consists of seminiferous tubules lying in a connective tissue stroma, each tubule being surrounded by a delicate basement membrane at the periphery of which are a few collagen and elastic fibres. The tubules are lined by Sertoli cells and spermatogonia which divide to produce spermatocytes and spermatozoa, the latter being present in the lumina of the tubules. Between the tubules lie the interstitial cells of Leydig which usually contain crystalloids together with the pigment lipofuscin and refractile granules.

This department has seen an exceptionally large number of testicular biopsies from subjects with chromatin-positive and chromatin-negative Klinefelter's syndrome, in both

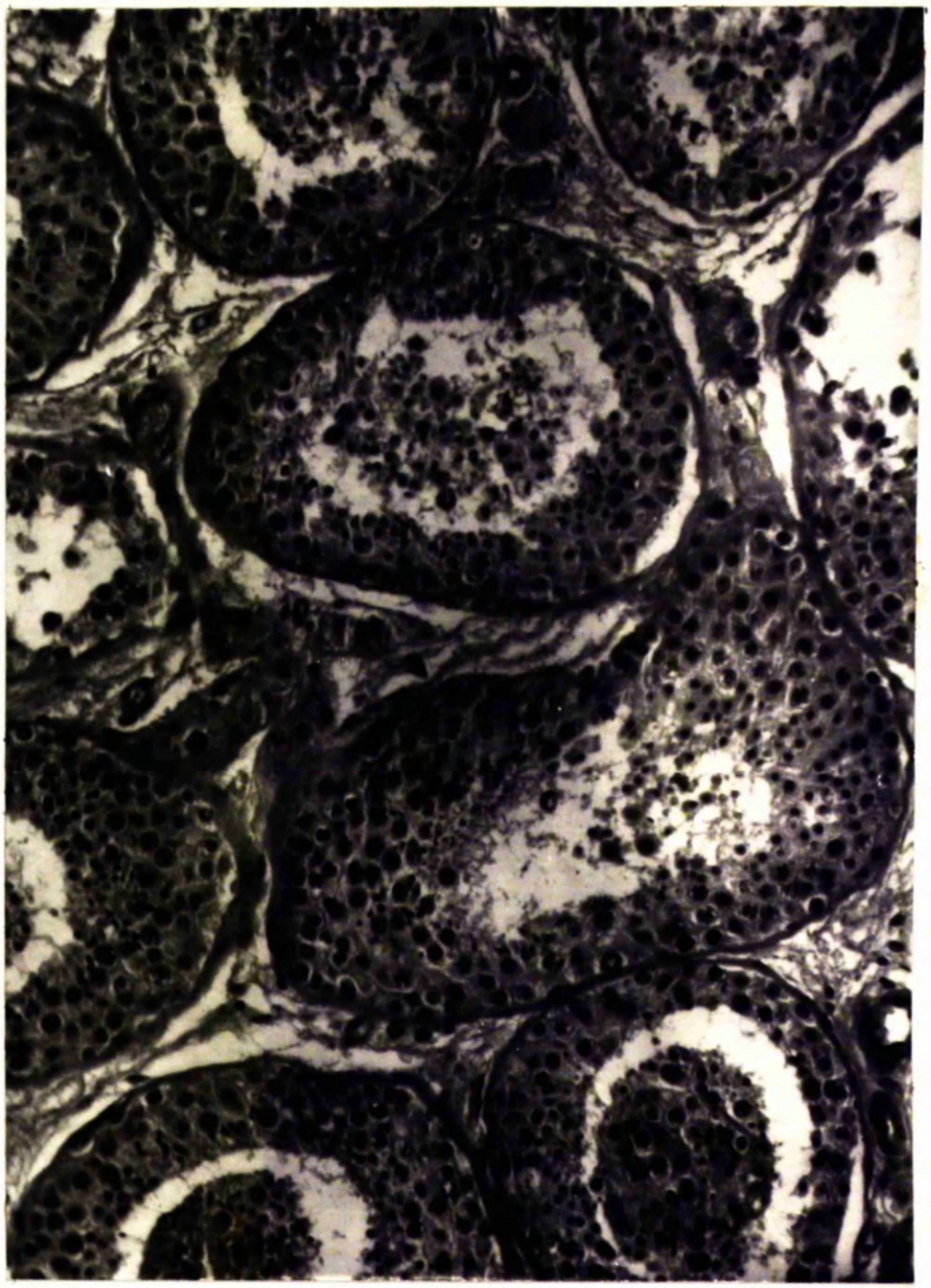

Figure 5 - Normal adult testis.
(Haemalum and eosin. X 240).

of which abnormal histological features are present. Nelson (1956) was the first to point out that a difference could be distinguished between the histology of the two forms and this has been confirmed by Ferguson-Smith et al. (1957), whose material I have been privileged to re-examine.

The testes in chromatin-positive Klinefelter's syndrome are smaller than normal and are of variable consistency. The normal histology is grossly disorganised (Fig. 6). The interstitial cells of Leydig are aggregated into solid, massive, discrete, well-vascularised, almost adenomatous-like clumps in which the individual cells show marked pleomorphism (Ferguson-Smith et al. 1957). Vacuolation is present, indicative of a degenerative process and there is a deficiency of refractile granules related to a functional failure (Barr, 1957).

The tubules are irregularly distributed throughout the testis, and large areas of testicular tissue may be devoid of tubules. The tubules themselves show varying degrees of fibrosis, over 70% of them, according to Lennox et al. (1958), showing hyalinisation. Many may be completely sclerosed and form 'ghost tubules'. Non-sclerosed tubules vary greatly in size, some reaching almost normal adult diameters, others being small and immature. These tubules show a small lumen or even none at all.

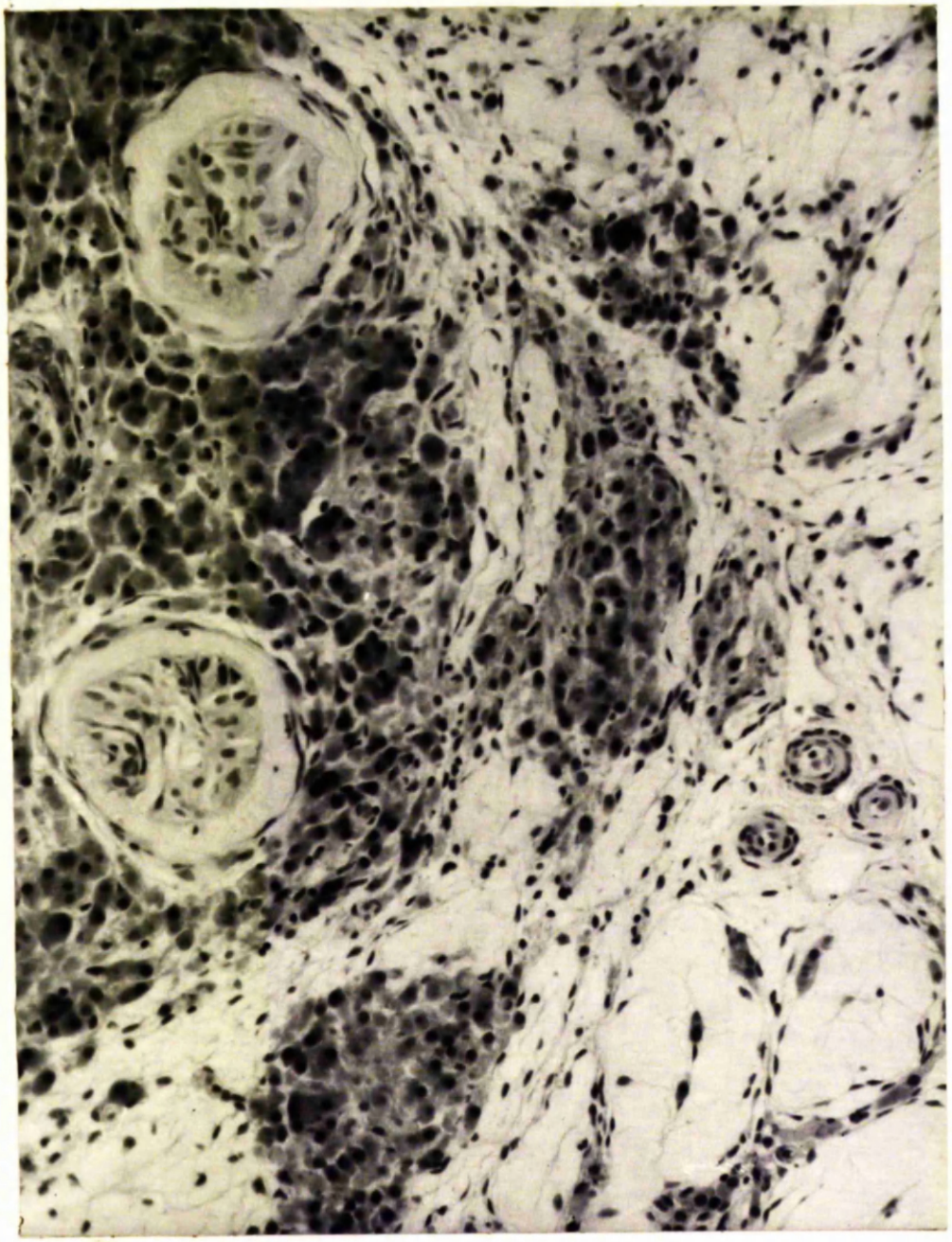

Figure 6 - The testis in chromatin-positive Klinefelter's syndrome showing clumps of Leydig cells and hyalinised tubules. (Haemalum and eosin. X 240).

A feature of note is the gross deficiency of elastic fibres. Elastic fibres usually appear in the testis around the age of 10 years, and Ferguson-Smith (1958), emphasised that in the hyalinised tubules of chromatin-positive Klinefelter's syndrome, the lack of elastic fibres indicates that the testicular lesion began before puberty.

In addition to the lesions just described, severe testicular atrophy with premature senility may be superimposed with the result that the remaining tubules atrophy to become 'ghost' tubules and the Leydig cells disappear leading to androgen deficiency (Ferguson-Smith, 1958).

Although hyalinisation of the tubules is seen frequently, it is not an invariable feature, and Ferguson-Smith (1958) observed that the most significant defect in the testis is a reduction in the number of germ cells. The tubules therefore are lined mostly by Sertoli cells which may be well preserved as long as the fibrosis in the tubules is minimal. (Siebenmann, 1958). Spermatogonia, as just mentioned, are rarely seen, but spermatocytes and even spermatozoa have been reported in several cases. (Bunge and Bradbury, 1956, Nelson, 1956, Lennox et al. 1958, Ferguson-Smith and Munro 1958). It is therefore theoretically possible for a case of chromatin-

positive Klinefelter's syndrome to be fertile, but, as previously noted, no such case has yet been proved unequivocally.

Before puberty, according to Overzier (1963b), the appearance is not quite as characteristic, but may be quite striking. Overzier states that the tubules may vary in diameter and are lined by very immature syncytial cells. Germ cells are reduced in number before puberty as well as in the adult (Ferguson-Smith 1959), and tubular hyalinisation does not occur until puberty (Ferguson-Smith 1959, Overzier, 1963b).

Chromatin-negative Klinefelter's Syndrome

This syndrome forms a reasonably homogeneous group, but one less well defined than chromatin-positive Klinefelter's syndrome, from which there are a few points of distinction. Stewart et al. (1959) analysed 16 chromatin-positive and 16 chromatin-negative cases of Klinefelter's syndrome. They concluded that, whereas both forms show significant differences from normal with regard to the size of the testis and prostate, excretion of follicle-stimulating hormone and distribution of body hair, there were certain differences between the two groups. Stewart et al. demonstrated that in the chromatin-positive form, the testes were slightly smaller than in the chromatin-negative

form, and that, in addition, whereas chromatin-negative patients show no measurements which deviate significantly from normal, the chromatin-positive subjects had an increase in their height and in the sole to pubis measurement. Stewart et al. also added that the chromatin-positive patients showed deviations from normal with regard to the amount of facial hair, the frequency of shaving, and the incidence of gynaecomastia.

Although the clinical diagnosis of chromatin-negative Klinefelter's syndrome is less certain than the chromatin-positive form, the testes in the two groups are distinguishable on histological grounds. Ferguson-Smith et al. (1957) and Lennox et al. (1958) have shown that in the chromatin-negative form, in contrast to the chromatin-positive form, the Leydig cell hyperplasia is diffuse and even, and the cells themselves appear normal (Fig. 7). The seminiferous tubules are well preserved, less than 30% being totally hyalinised. Most of the tubules are lined by Sertoli cells, but total lack of germ cells is unexceptional and a few spermatogonia are usually seen. Few tubules are 'ghost' tubules, and there is no gradation between these and the tubules lined by Sertoli cells. Compared with the appearance in chromatin-positive Klinefelter's syndrome, the tubules are more uniform and the ghost tubules, which are

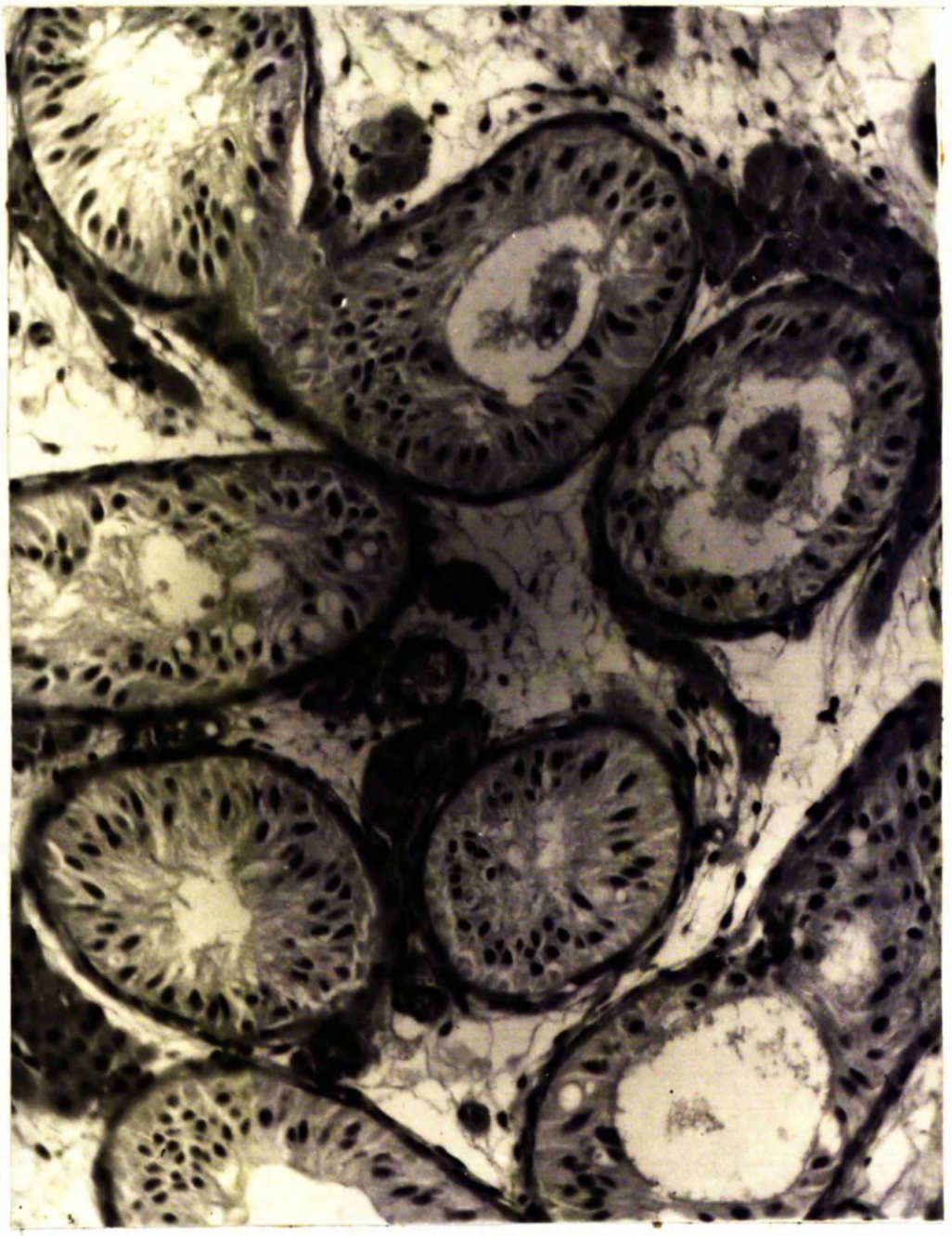

Figure 7 - The testis in chromatin-negative Klinefelter's syndrome showing well preserved tubules lined largely by Sertoli cells.
(Haemalum and eosin. X 240).

rich in elastic tissue, are larger (Polani 1962). As previously mentioned, elastic fibres usually appear in the testis around the age of 10 years, and consequently, the presence of elastic tissue indicates that the lesion is post-pubertal.

The features of chromatin-negative Klinefelter's syndrome were described in 1947 by del Castillo and his colleagues, who postulated that this new syndrome was due to the failure of migration of germ cells to the primary gonad. Although some cases of chromatin-negative Klinefelter's syndrome may be regarded as a primary failure of germ cell development, a less well-defined group exists, in which the damage to the testis may be a result of exogenous factors such as inflammation, trauma, interference with the vascular supply, etc.

Association with Infertility

As can readily be predicted from the testicular morphology, Klinefelter's syndrome shows a marked association with infertility. In 1957, Ferguson-Smith and his colleagues investigated patients attending a male infertility clinic, and found that 8.5% of all cases of male subfertility, and 30% of cases with major impairments are due to Klinefelter's syndrome (both chromatin-positive and chromatin-negative). 40% of these

cases with Klinefelter's syndrome were found to be chromatin-positive. Consequently, Ferguson-Smith et al concluded that 11% of cases of high grade subfertility and 3% of all cases of subfertility in males are due to chromatin-positive Klinefelter's syndrome.

The frequency of chromatin-positive Klinefelter's syndrome

In 1959, a leading article in the 'Lancet' showed that by combining the figures for the incidence of infertility in the general population, of azoospermia in infertility, and of chromatin positive Klinefelter's syndrome in azoospermics, it could be estimated that chromatin positive Klinefelter's syndrome occurred in at least 1 in 600 of the general population.

Since then there have been several surveys of male and female newborn babies by the buccal mucosal smear method of nuclear sexing, in order to estimate the incidence of sex chromatin anomalies. With regard to the male babies, Moore (1959) found 5 cases with chromatin-positive nuclei in 1911 babies, Bergemann (1961b) found 4 cases in 1890, Maclean et al. (1961) found 9 cases in 3,000, and Subray and Prabhaker (1962) working in India, found none in 2058 male babies. Wiesli,

working in Basle, studied amnion cells of 1563 newborn males and found sex chromatin in only one case which was an anencephalic.

Maclean et al. (1964) have extended their previous 1961 survey, and have recently reported a further 12 cases in 7,725 babies, making a total from the Edinburgh group of 21 cases in 10,725 liveborn male babies.

By combining the figures from the various groups of workers and excluding the anencephalic case reported by Wiesli, 30 cases of presumptive chromatin-positive Klinefelter's syndrome have been found in 18,147 male babies, i.e. 1.65 per thousand, or 1 in 605. This incidence is similar to the frequency of 1 in 600, which, as shown above, had been previously calculated by indirect means. It is, however, to be noted that in the largest survey of the group, which was performed by Maclean and his colleagues, the incidence is 1.96 per thousand, and that if only the surveys performed by Moore, Bergemann and Maclean are taken into account, the frequency of chromatin-positive Klinefelter's syndrome in the general population is 2.07 per thousand.

Association of chromatin-positive Klinefelter's syndrome with mental deficiency.

There is a well marked association between mental deficiency and chromatin-positive Klinefelter's syndrome, and, indeed, mental deficiency was present in one of the original cases described by Klinefelter and his colleagues in 1942. In 1957, Pasqualini et al. drew attention to the fact that 11 of 31 patients with clinical features of Klinefelter's syndrome whom he examined were feeble-minded, but in none of these patients had nuclear sexing been performed. In the same year, Ferguson-Smith and his colleagues, as previously mentioned, investigated patients attending a male infertility clinic, and in addition to observing the association between Klinefelter's syndrome and infertility, also noted that some of the chromatin-positive patients were of low intelligence. These observations rapidly led to several surveys of mental defectives, the results of which are shown in Table 1. Combination of these figures shows that the incidence of males with chromatin-positive nuclei is 132 in 14239 male mental defects, or 9.3 per thousand. Ten of these 132 chromatin-positive subjects were reported to have more than one sex chromatin body in some of their nuclei.

Table I

Frequency of Chromatin-Positive Klinefelter's Syndrome in Mental Defectives

Type of Survey	Author	No. Cases	No. Chromatin Positive Cases	% Incidence
Schools for Educationally Subnormal Children	Prader et al. (1958)	336	8 (1)	2.4
	Ferguson-Smith (1959)	633	8 (1)	1.2
	Cornwell (1960)+	409	3 (1)	0.73
	Chapelle & Hortling (1960)	342	3 (1)	0.88
	Israelsohn & Taylor (1961)	1556	7 (1)	0.45
		3276	29	0.89
Mental Institutions	Ferguson-Smith (1958)	325	4 (1)	1.2
	Mosier et al. (1960)	1252	10 (1)	0.8
	Barr et al. (1960)	1506	11 (1), 3 (2)	0.86
	Shapiro & Ridler (1960)	900	6 (1)	0.66
	Sanderson & Stewart (1961)	245	2 (1)	0.82
	Gustavson & Åkesson (1961)	48	0	0
	Hamerton et al. (1962)	229	1 (1)	0.44
	Maclean et al. (1962)	2607	23 (1), 4 (2), 1 (3)	1.07
	Ferguson-Smith (1962)	916	7 (1), 2 (2)	0.98
	Davies (1963)	550	3 -	0.73
	Forssman & Hambert (1963)	760++	15 (1)	1.97
		1625	10 (1)	0.62
		10963	103	0.94
	Overall Incidence	14239	132	0.93

( ) No. sex chromatin bodies present

\+ Cited by Israelsohn and Taylor (1961), as unpublished results.

++ 'Criminals or hard-to-manage males.'

However, not all cases of chromatin-positive Klinefelter's syndrome are necessarily mentally defective. Of the subjects with chromatin-positive Klinefelter's syndrome who were found in a male infertility clinic, Ferguson-Smith (1959) said, "Certainly no more than a quarter of these patients showed signs of subnormal intelligence and some appeared to have more than average intelligence". Both Ashley and Overzier in their reviews mention that they have seen cases in patients with average or above average intelligence.

In order to determine whether chromatin-positive Klinefelter's syndrome is of more frequent occurrence in patients with the less severe forms of mental deficiency, Israelsohn and Taylor (1961) compared the results of various surveys which had then been carried out. These surveys had been performed both on children with the milder degrees of mental deficiency who were attending schools for the educationally subnormal, and on patients with more severe forms of mental deficiency who were to be found in mental institutions. Israelsohn and Taylor concluded that the only evidence of a higher incidence of chromatin-positive Klinefelter's syndrome in subjects with less severe degrees of mental deficiency was the

increased frequency reported in educationally subnormal schoolchildren by Prader and his colleagues (1958).

Comparison of the surveys which have been made to date show an incidence of 8.9 per thousand in educationally subnormal schoolchildren compared with 9.4 per thousand in patients in institutions for mental defectives. However, a feature of note is the relatively high incidence of 15 chromatin-positive males in 760 patients (or 19.7 per thousand) reported by Forssman and Hambert (1963), who surveyed three hospitals for "criminal or hard-to-manage males of subnormal intelligence", in whom the degree of mental deficiency was not reported. Inclusion of the results of this survey with those obtained from other mental institutions produces the relative frequencies mentioned above. If, however, these patients are regarded as having a less severe degree of mental deficiency, then this relatively high incidence of 19.7 per thousand reported by Forssman and Hambert would tend to support the suggestion that chromatin-positive Klinefelter's syndrome is commoner in the less severe forms of mental defect. In addition, the overall incidence of chromatin-positive males in the milder forms of mental deficiency becomes 10.7 per thousand and the frequency in the more severe forms becomes 8.6 per thousand.

These findings are discussed in some detail since we shall consider later whether the degree of mental defect in cases of Klinefelter's syndrome who have more than one sex chromatin body in their nuclei, (i.e. the XXXY and XXXXY chromosomal variants), is similar to that in the commoner variety of the syndrome.

Aetiology of chromatin-positive Klinefelter's syndrome

When Klinefelter's syndrome was first described in 1942 by Klinefelter, Reifenstein and Albright, the authors suggested that it was due to a primary failure of testicular function, which, by producing a hormonal imbalance between the testis and the pituitary, accounted for the clinical signs and symptoms. When, however, in 1956, it was found that some of the subjects with the features of Klinefelter's syndrome had chromatin-positive nuclear sex, the situation appeared more complicated, and there was considerable speculation with regard to the aetiology of this form of intersex.

It was postulated that the nuclear sex corresponded to the chromosomal sex complex of XX (e.g. Danon and Sachs 1957), and patients with chromatin-positive Klinefelter's syndrome were regarded as 'genetic females' who had undergone 'sex

reversal' in foetal life. Various embryogenetic mechanisms were suggested to account for this phenomenon. For example, it was suggested that testes developed in these 'genetic females' as a result of cortico-medullary imbalance in gonads destined to become ovaries. This cortico-medullary imbalance was thought to be due either to the failure of cortical development (Bunge and Bradbury 1956, Grumbach et al. 1957), or to reinforcement of the influence of the medulla by some external influence (Segal and Nelson 1957). Witschi and his colleagues (1957) offered an alternative suggestion that the involution of the cortex was the result of an impairment in the number of germ cells reaching the gonad.

As I have just mentioned, it was believed that the presence of sex chromatin in subjects with chromatin-positive Klinefelter's syndrome indicated that the sex chromosome complex contained two X chromosomes. This assumption was reinforced by the low frequency, in these patients, of colour blindness (Polani et al. 1958), which is carried on a recessive gene on the X chromosome, and tends to be manifested when only one X chromosome is present. When Ford and his colleagues (1959c) and Jacobs and Strong (1959) examined the chromosomes of these chromatin-positive patients, they found that, although

two X chromosomes were indeed present, there were 47 instead of 46 chromosomes and the complete sex chromosome complex was XXY. This observation has been confirmed by numerous workers cited by Polani (1962).

The origin of the XXY chromosome anomaly

As previously mentioned in part 1, numerical abnormalities of chromosomes are believed to be the result of non-disjunction occurring during meiosis or mitosis. The XXY chromosome anomaly could therefore arise if primary non-disjunction occurs in either the sperm or the ovum, resulting in either fertilisation of a normal X-bearing ovum by a non-disjoined XY sperm, or fertilisation of a non-disjoined XX ovum by a normal Y sperm.

The XXY anomaly could also theoretically be the result of secondary non-disjunction, which is said to occur when an abnormal number of chromosomes is found in the gametes of individuals who already possess an abnormal chromosomal complement. Thus, in theory, an XXY zygote can arise from secondary non-disjunction in an XXY father, or in a mother who possesses the sex chromosome pattern of XXX. However, as previously mentioned, fertility in an XXY Klinefelter male has

been difficult to prove, and with regard to the XXX anomaly (which will be discussed in part 5), all the children of XXX mothers have been shown to have normal nuclear sex or normal sex chromosome complexes. For these reasons, therefore, primary non-disjunction is the more likely cause of the XXY anomaly.

Another possible mode of origin of the XXY abnormality is by mitotic non-disjunction occurring in the early cell division of a normal XY zygote with elimination of the nonviable YO cell line. This method of origin is discussed by Ford (1963), who observes that if the XXY anomaly arose from non-disjunction of the XY zygote, the frequency of colour blindness in chromatin-positive Klinefelter's syndrome would be the same as in normal males. Since, however, colour blindness is of much lower frequency in these patients, as Ford points out, most XXY Klinefelter cases must therefore possess two genetically distinct X chromosomes derived from non-disjunction of the gametes.

In order to determine whether maternal or paternal non-disjunction is the cause of the sex chromosome anomaly, further studies have been made on families of colour blind subjects with chromatin-positive Klinefelter's syndrome by

Nowakowski et al. (1959), and similar studies have been carried out on cases of chromatin-negative gonadal dysgenesis, e.g. by Stewart (1959). The results of Nowakowski and his colleagues show that in the colour blind Klinefelter cases which they investigated, the two X chromosomes were of maternal origin. Using evidence from colour blindness studies alone, however, it would be difficult to obtain proof of paternal non-disjunction as the cause of the XXY anomaly, since as Lennox (1961) and Polani (1961b), have both pointed out, this would necessitate the investigation of a family containing a non-colour blind XXY male with a colour blind mother and a normal father. These cases would be found only rarely.

Recently, however, the sex-linked blood group antigen Xg^a, described by Mann et al. (1962), has been used by Frøland et al. (1963) to investigate the origin of the two X chromosomes in the XXY complex. In three of four families containing chromatin-positive Klinefelter subjects, these workers have shown that the two X chromosomes were of maternal origin. In the fourth family, however, they have demonstrated that one of the X chromosomes was of paternal, and the other, of maternal origin, i.e. that paternal non-disjunction in this particular instance had caused the XXY anomaly. This observation has since been

confirmed by Ferguson-Smith et al. (1964).

In passing, it may be noted that with regard to the origin of the XO anomaly in chromatin-negative ovarian dysgenesis, although the results of colour blindness studies, e.g. by Stewart (1959), offered evidence that the single X chromosome was of maternal origin, investigation of the Xg^a blood group antigen by Lindsten et al. (1963) has shown that the X chromosome may be either of maternal or paternal origin, i.e. that the XO anomaly may be caused by either maternal or paternal non-disjunction.

Although the XXY sex chromosome complex is the most frequently encountered form of chromosomal anomaly in chromatin-positive Klinefelter's syndrome, several other chromosomal variants have been described including mosaics.

It has been found that in a few subjects with this syndrome, the sex chromosome complex has included more than two X chromosomes, so that in addition to the usual XXY form, XXXY and XXXXY sex chromosome variants have been described. I have recently had the opportunity of examining two patients, one with the XXXY anomaly, and the other with the XXXXY anomaly, and since the clinical features of these patients serve to demonstrate the effect in the male of increasing numbers of X

chromosomes, the XXXY and XXXXY chromosomal variants will be considered separately.

The XXXY sex chromosome anomaly

A total of 6 subjects with the XXXY sex chromosome complex have been described by Barr et al. (1959), Ferguson-Smith et al. (1960a), and Carr et al. (1961c). These patients were found by nuclear sex surveys of mental defectives in institutions, and were detected because their buccal mucosal smears contained two sex chromatin bodies in some of the nuclei.

Nuclear sexing has revealed a further case of the XXXY variant in a 40 year old mentally defective patient, who, after admission to Stirling Royal Infirmary, was noted to show some features of Klinefelter's syndrome.

On physical examination, the subject is of average height, (69 ins. or 175.3 cms.), and has slightly long legs, the pubis to sole measurement being 35½ ins. (89.54 cms.). The patient is heavily built and has a somewhat feminine distribution of fat. The hips are broad, the abdomen obese, and slight gynaecomastia is present. Axillary and pubic hair is scanty and the pubic hair is of feminine distribution. Chest and

facial hair are absent. There is no evidence of acne on the face or body, and there are no ocular defects such as myopia or strabismus. The voice is high pitched.

Abnormalities are present in the external genitalia, for although the scrotum is normally formed, the penis is underdeveloped. The testes are not palpable in the scrotum or groin, and are apparently intra-abdominal.

There is also evidence of skeletal abnormalities shown by marked limitation of pronation and supination of both forearms. Some degree of limitation of inversion is present at the ankle joints but passive eversion is possible.

The patient is of subnormal intelligence and lives at home with a sister. Formal testing in order to assess the intelligence quotient could not be carried out, but it was noted that the patient is quiet, cheerful and co-operative, although slow in performing tasks; he can feed and dress himself, can answer simple questions, and works as a road-sweeper.

At the time of the patient's conception, both parents were 37 years of age. The subject is the second of a sibship of four. The youngest sib, a female, died in infancy from meningitis. The other two sisters are healthy and have normal intelligence. One of these sisters is married and has an

apparently normal family.

Unfortunately, apart from a physical examination and the collection of peripheral blood for chromosomal analysis together with smears of the buccal mucosa, the patient's relatives would not give permission for any further investigations.

Sex chromatin and chromosome investigations.

The buccal smears were fixed in Papanicolaou's fixative and stained with cresyl echt violet according to the method described by Moore and Barr (1955a). Examination of suitable nuclei showed that 22% contained 2 sex chromatin bodies, 60% contained 1 sex chromatin body, and 18% contained no sex chromatin.

Mitotic chromosome counts were made on cultured peripheral blood by the method of Moorhead et al. (1960). 81 of the 100 cells examined contained 48 chromosomes, 14 contained 47 chromosomes, 4 contained 46 chromosomes, and 1 contained 49 chromosomes. Analysis of cells containing 48 chromosomes showed that there were 22 pairs of autosomes together with a Y chromosome and 3 extra chromosomes lying in the medium size range (Fig. 8). On the basis of the sex chromatin

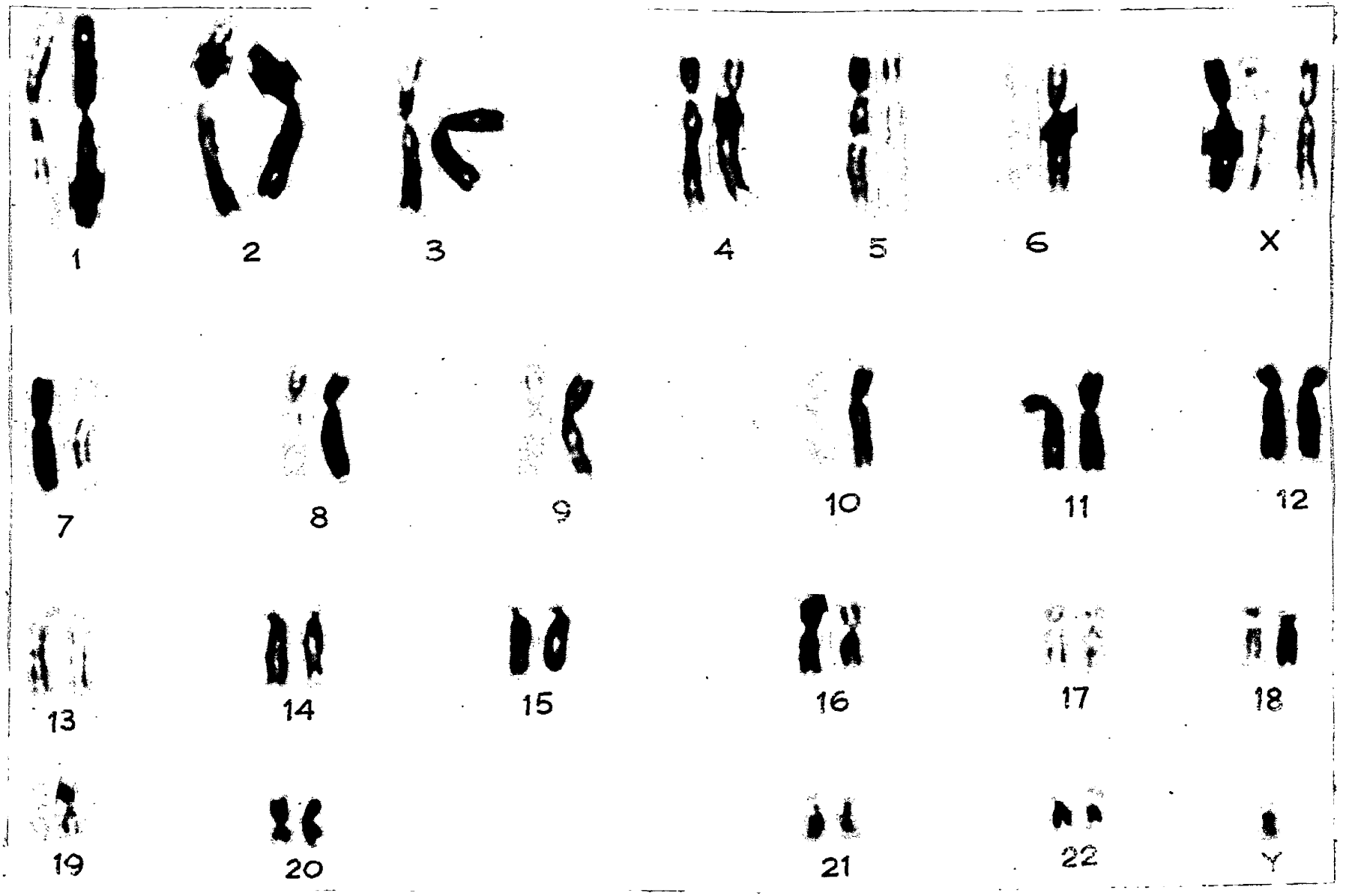

Figure 8 - Karyotype showing 22 pairs of autosomes together with the Y chromosome and the three presumptive X chromosomes, numbered according to the "Denver" system (1960).

findings, these extra chromosomes were assumed to be X chromosomes with the resulting sex chromosome pattern of XXXY. Of the 14 cells containing 47 chromosomes, 7 were thought to have the XXY sex chromosome complex, and although the percentage of XXY cells is small, the possibility of mosaicism in this patient cannot be completely excluded.

Comparison of patients with the XXXY anomaly

It is interesting to compare the clinical features of subjects with chromatin-positive Klinefelter's syndrome in whom the sex chromosome complex differs from XXY, in order to ascertain whether these chromosomal variants form specific syndromes which can be clinically recognised. For this purpose, details of the seven patients with the XXXY, (or presumptive), chromosome anomaly are shown in Table 2. Analysis of the data shows no outstanding features common to all the patients which might form a specific syndrome distinct from the XXY variant, but as indicated below, a few minor abnormalities are present in some of the cases.

It can be seen that features of the usual XXY form of chromatin-positive Klinefelter's syndrome are present in various patients of the group, including, for example, increased height

TABLE 2. XXY KLINEFELTER'S SYNDROME

Author	Case No.	Age	I.Q.	Height (cms.)	Span (cms.)	Pubis to Sole (cms.)	Incidence of Drumsticks	% Sex Chromatin Single	% Sex Chromatin Double	Maternal age	Paternal Age	External Appearance	Genitalia	Histology of Gonads	Skeletal Abnormalities	Other Features
Barr et al., (1959)	1	55	54	184	179	98	6/230	47	25	37	37	Long legs. Facial hair scanty.	Penis small. Testes v. small.	Consists almost entirely of Leydig cells. One tubule seen with thickened tunica propria. No germ cells.	-	Died. Chromosomes not analysed.
	2	31	23	174	172	89	1/2800	31	33	38	28	Normal habitus. No gynaecomastia. Facial & pubic hair present.	Penis and testes normal.	Nearly normal. Some arrest of maturation & scarcity of sperms.	-	Chromosomes not analysed. Gonadotrophins and 17-ketosteroid excretion normal.
Ferguson-Smith et al., (1960)	3	22	14	171	164	93	6/433	42	38	38	52	Thin. Asthenic. Small head. Long legs. Myopic. Lumbar lordosis. Webbed neck. Partially erupted teeth. No gynaecomastia. Facial, axillary & abdominal hair scanty.	Penis & scrotum normal. Prostate small. Testes v. small.	Resembles XXY testis.	Bilateral radio-ulnar synostosis.	-
	4	22	<20	175	177	94	6/725	36	35	27	31	Long legs. Bilateral gynaecomastia. Facial hair scanty.	Penis & scrotum adolescent. Testes v. small & high up in scrotum Prostate small.	Resembles XXY testis	-	-
Carr et al., (1961c)	5	15	60	166	166	86	6/307	41	32	21	41	Normal habitus. No gynaecomastia.	Penis & testes small.	Resembles XXY testis	-	Low B.M.R. Deficient I^{131} uptake by th
	6	14	60	162	162	88	3/500	33	42	20	23	Normal habitus. Sl. gynaecomastia	Penis & testes small	Resembles XXY testis	-	Cephalin cholesterol Thymol turbidity } Abnor
Present Case	7	40	M.D.	175	175	90	-	60	22	37	37	Long legs. Sl. gynaecomastia. Facial & chest hair absent. Axillary & pubic hair scanty.	Penis small. Scrotum normal. Testes undescended.	-	Limited supination & pronation of both forearms. Limited inversion of both feet.	-

long legs, gynaecomastia, poor growth of facial and body hair, small testes and penis.

In four of the six cases in whom the gonads were examined histologically, the appearances, at least from all the descriptions given, are similar to the testis in the XXY variant of Klinefelter's syndrome. The remaining two cases, however, which are both described by Barr et al. (1959) show a somewhat different histological appearance. In one of these subjects, (case 1), the testis is described as consisting almost entirely of Leydig cells. Only one tubule was seen: this contained no germ cells but had a thickened tunica propria. The other subject, (case 2), was a mentally defective male in whom there were no clinical or hormonal features of Klinefelter's syndrome. The testes of this patient were nearly normal on histological examination, the only minor abnormality being some arrest of maturation and scarcity of spermatozoa. Barr and his colleagues (1960), in a later paper, say of this patient that he "cannot be included in Klinefelter's syndrome, for his testes were normal in size and histological structure". However, the presence of two sex chromatin bodies in some nuclei of the buccal mucosa indicates that at least some tissues or cell lines contain three X chromosomes (XXXY), but, unfortunately,

chromosome studies are not reported on this possible mosaic.

Evidence of skeletal abnormalities involving the forearms is present in two of the seven patients under consideration. In the present case, there is limitation of supination and pronation of both forearms, and in one of the cases described by Ferguson-Smith et al. (1960a), there is bilateral radio-ulnar synostosis. This latter case also shows some other minor congenital abnormalities including neck webbing and myopia.

The presence of malfunction of the thyroid and liver in cases 5 and 6 is of interest, since disordered function of the liver has also been described in an XXYY variant of Klinefelter's syndrome by the same group of workers (Carr et al. 1961b). Barr and his colleagues (1960) noted that thyroid abnormalities were present in a group of, (presumably XXY), chromatin-positive Klinefelter's patients with mental deficiency, and suggested that underactivity of the thyroid gland during the developmental period may be a factor contributing to the mental retardation. That this deficiency may be the result of a thyroiditis is suggested by the fact that the latter is known to be the case in instances of chromatin-positive Turner's syndrome with the X isochromosome-X constitution (Sparkes and

Motulsky 1963).

All seven cases of the group are mentally retarded. As previously noted, 6 of these 7 cases were detected on nuclear sexing surveys of mental defects in institutions, and these cases show a fairly severe degree of mental defect, the I.Q. varying between 60 and 14. The number of cases concerned is too small for statistical analysis, but this range of I.Q. suggests that the degree of mental defect is, on the whole, greater than that encountered in the XXY variant, (see p. 112)

On the whole, therefore, the only features presented by these patients with the XXXY anomaly, which differ from the usual form of XXY Klinefelter's syndrome found in mental institutions, are the slightly greater degree of anomaly present in some patients and the occasional association of other congenital abnormalities.

The origin of the XXXY anomaly

The origin of the XXY anomaly has already been discussed. In the case of the XXXY abnormality, the situation becomes slightly more complicated and several possibilities arise. For example, firstly, primary non-disjunction might occur in both parents, followed by fertilisation of an XX ovum by an XY

sperm, but this possibility is highly unlikely on statistical grounds. Secondly, it is also unlikely, for reasons which have previously been mentioned, that the anomaly is caused by secondary non-disjunction occurring in an XXX mother or an XXY father giving rise to XXX and XXY gametes. An additional point against this explanation is the presence of normal nuclear sex in the mothers of the two cases described by Ferguson-Smith et al. (1960a). A third possibility is the occurrence of mitotic non-disjunction at the first cleavage division of an XXY zygote producing XXXY and XY cells, with elimination of the XY cell line. This, however, is improbable, for it would appear more likely that the normal XY cells would survive in preference to the abnormal XXXY cells. It would appear, therefore, that a fourth and most probable explanation of the XXXY anomaly would be the occurrence of primary non-disjunction at both meiotic divisions in either of the parents, producing an XXX ovum or an XXY sperm. These, when combined with a Y sperm or an X ovum respectively, produce an XXXY zygote.

The XXXXY chromosomal variant

In addition to the case which I am about to describe, there are nine other cases of XXXXY chromatin-positive

Klinefelter's syndrome. These were reported by Fraccaro and his colleagues, (i.e. Fraccaro et al. 1960c, and Fraccaro and Lindsten 1960), together with Anders et al. (1960), Miller et al. (1961), Fraccaro et al. (1962b), Pfeiffer (1962), Turpin et al. (1962), and Schade et al. (1963), all of whom reported one case. Barr and his colleagues however, described two cases in 1962.

The present case is a male child who at 8½ years of age was admitted to the Royal Hospital for Sick Children in Glasgow for interval appendicectomy. He is the oldest of a sibship of three, and at the time of his conception, his mother was 22 and his father 24 years of age. No significant findings arise in the family history.

The child who is 42 ins. (107 cms.) in height, is smaller than his stated age would suggest. Apart from the genitalia, the external appearance is unexceptional, (Fig. 9). The penis is normal, but the scrotum is underdeveloped and the testes undescended, (Fig. 10).

There is a severe degree of mental deficiency. When tested on the Merril-Palmer scale, at the age of 8 years 3 months, the mental age of the child was found to be 2 years 3 months, (i.e. an I.Q. of 33). The child is ineducable. Speech is very

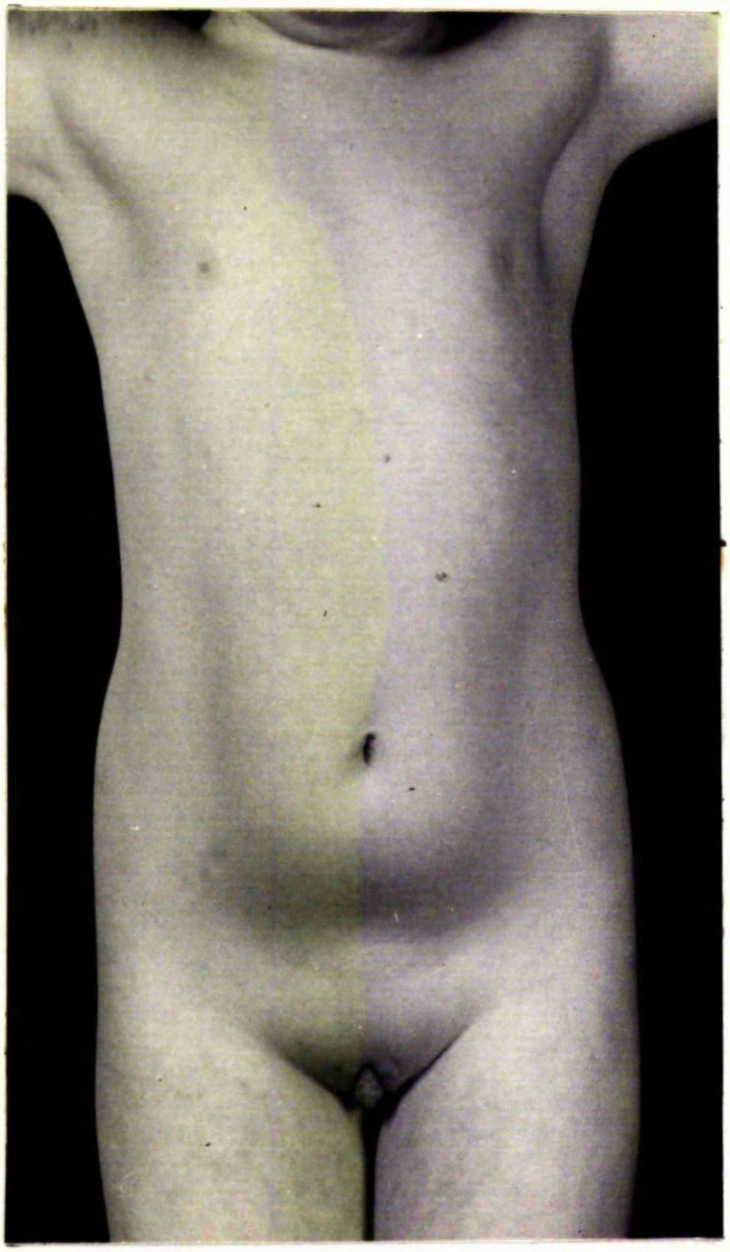

Figure 9 - External appearance of the XXXXY subject showing the underdeveloped scrotum.

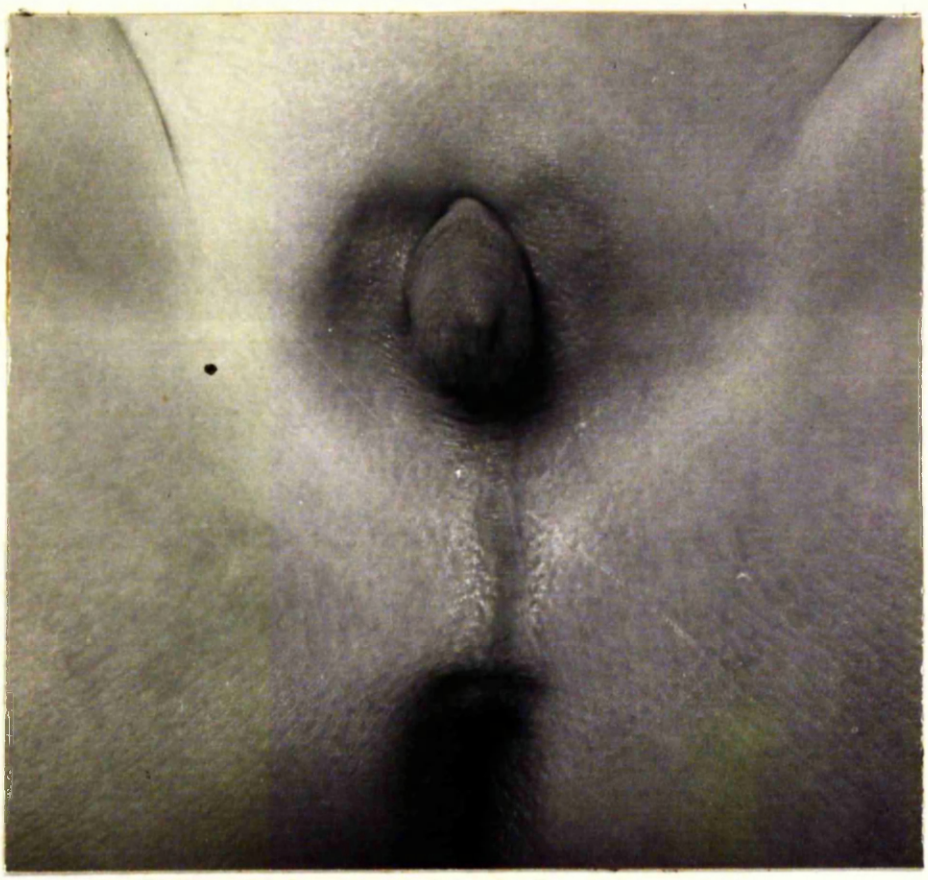

Figure 10 - External genitalia of the XXXXY subject showing the hypoplastic scrotum and normal penis. The testes are intra-abdominal.

indistinct, but this is probably due to a cleft palate which was repaired at the age of 3. A small perforation is present in both ear-drums, and it is possible that a slight degree of deafness may be impairing speech.

Radiological Examination

All the bones are small, but the centres of ossification are within normal limits for the age. Multiple bony abnormalities are present. The sella turcica is enlarged and deep, but the skull is otherwise normal (Fig. 11). There is bilateral coxa valga (Fig. 12). The ulnae are very slender and the proximal parts are expanded, particularly on the left side. The radius on both sides proximally is overdeveloped, and there is a radio-ulnar synostosis on the left side (Fig. 13). In each hand, there is an abnormal centre of ossification at the base of the second and fifth metacarpals. The middle phalanx of the little fingers contains two centres of ossification and the terminal phalanx in each little finger is curved. (Fig.14).

Testicular lesion

At laparotomy the appendix was removed and both testes were found in the pelvis near the internal inguinal ring.

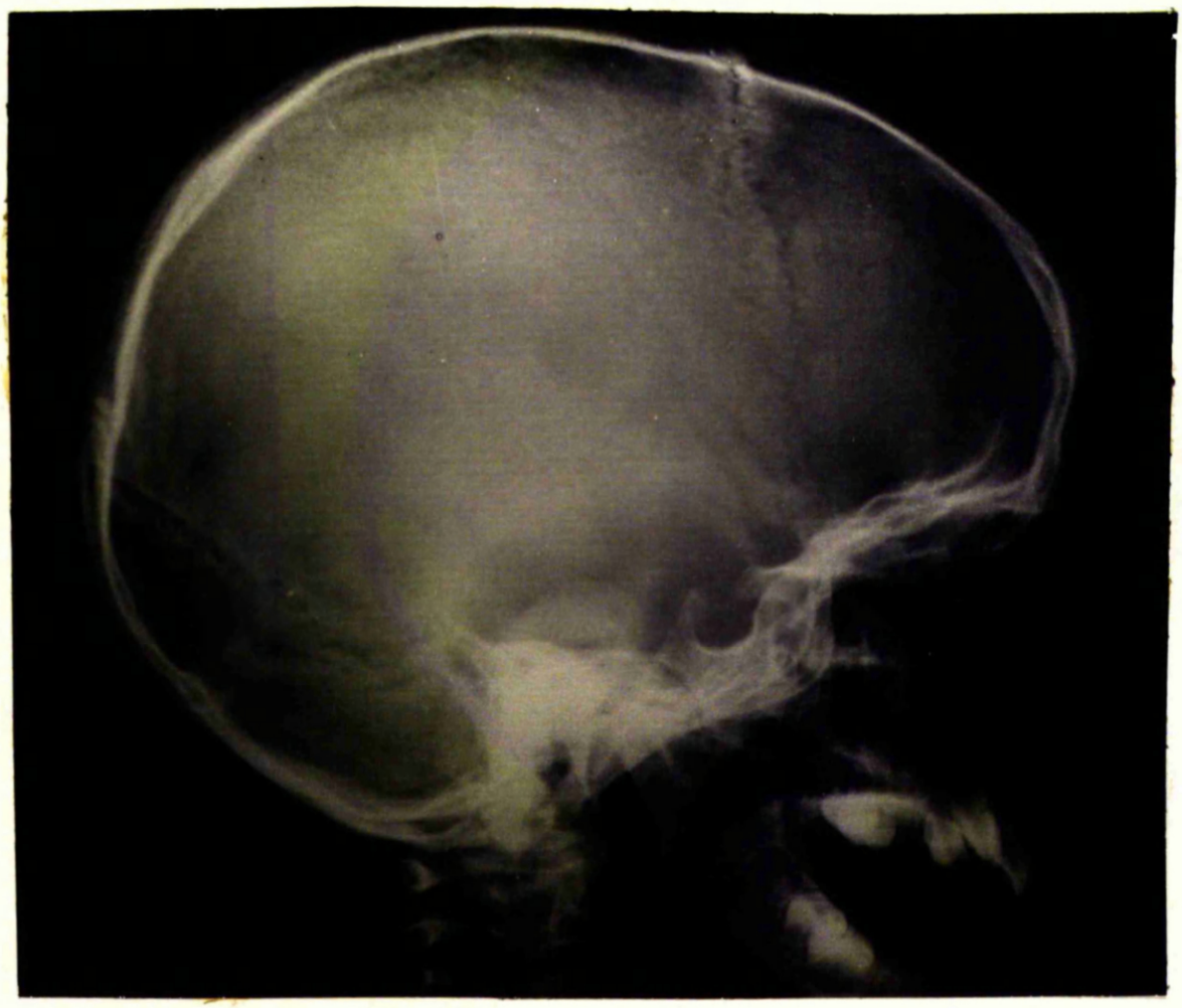

Figure 11 - X-ray of skull showing the enlarged and deep sella turcica.

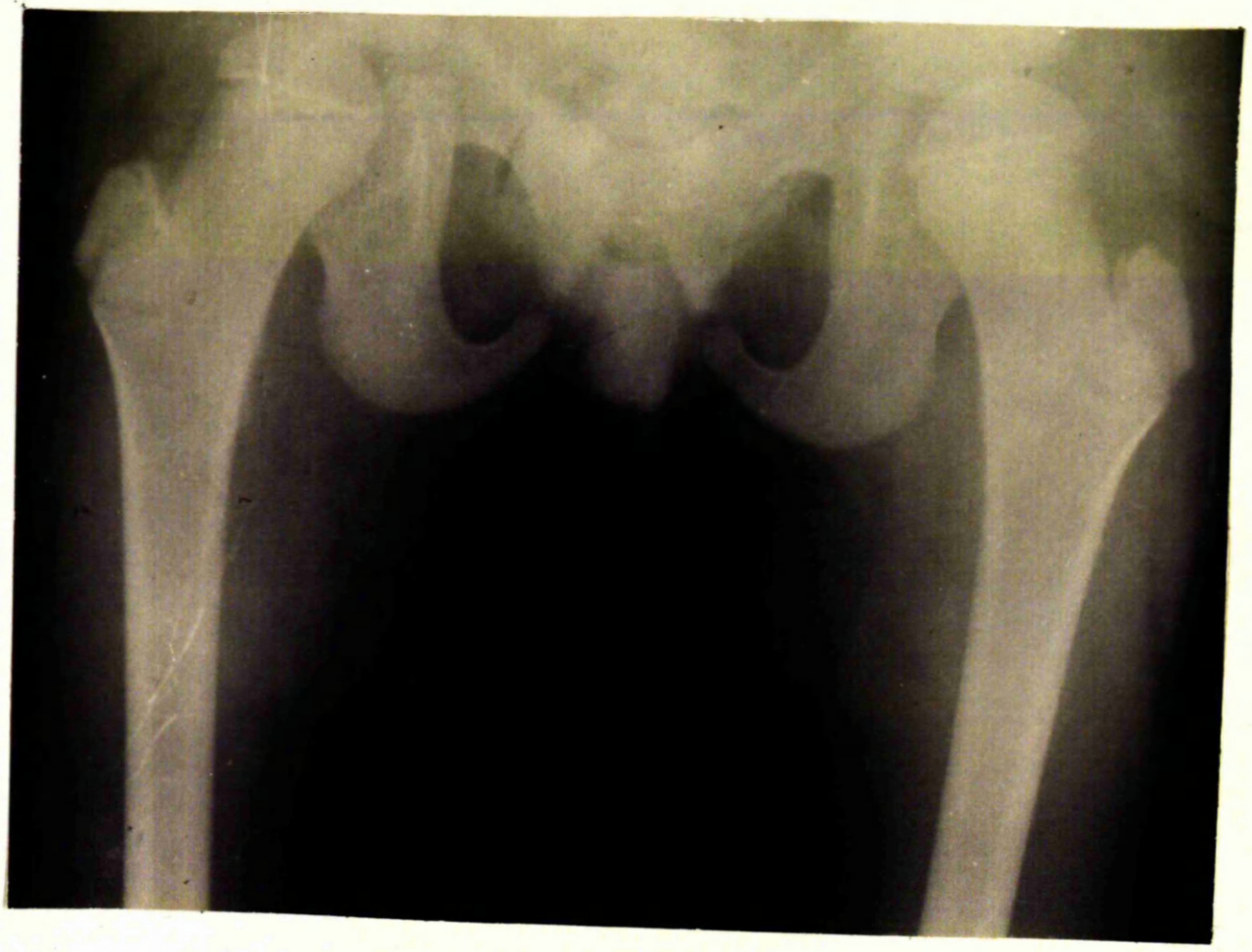

Figure 12 - X-ray of femora and pelvis showing bilateral coxa valga.

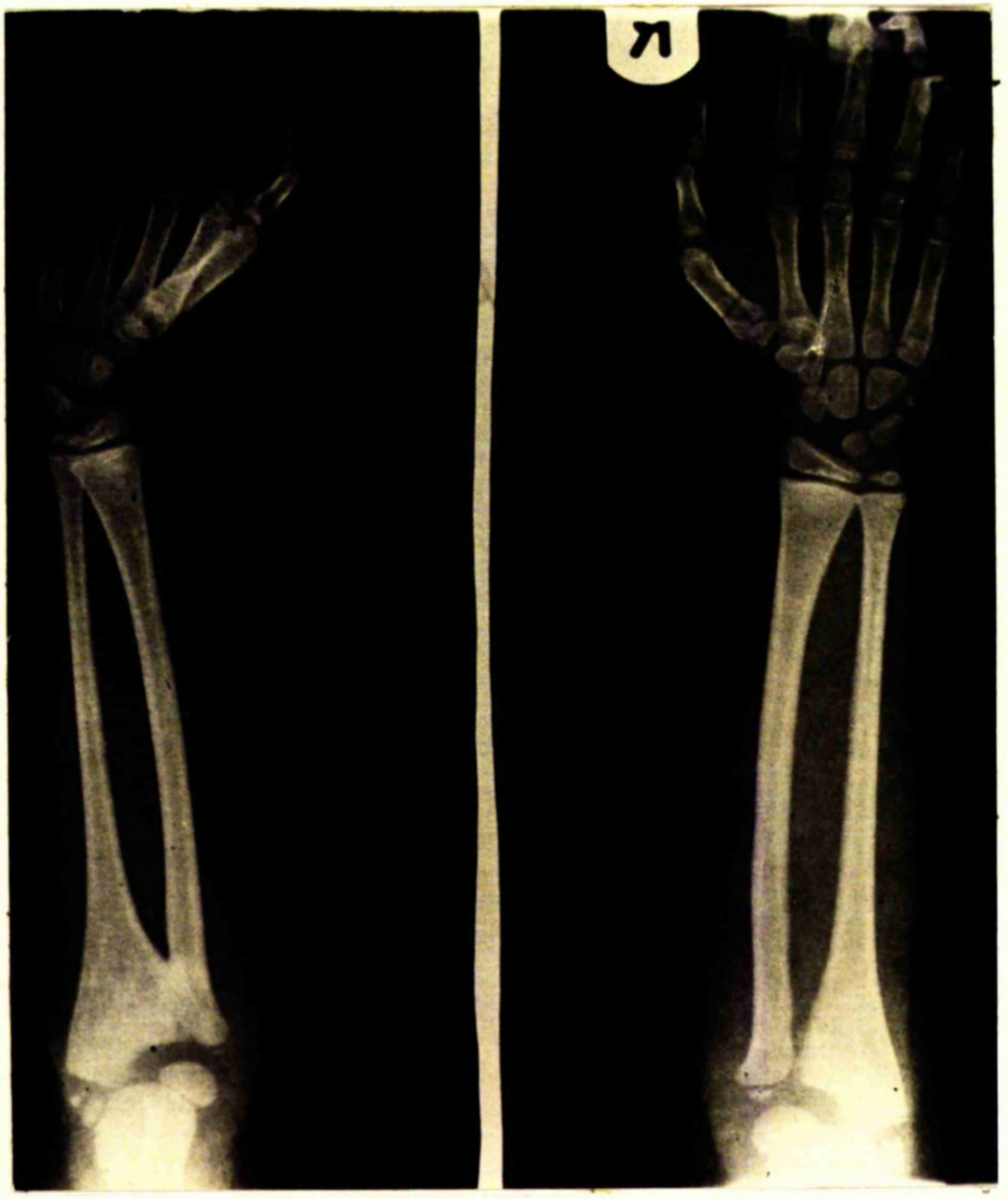

Figure 13 - X-ray of forearms and elbow-joints showing a radio-ulnar synostosis on the left side and other deformities of the radius and ulna.

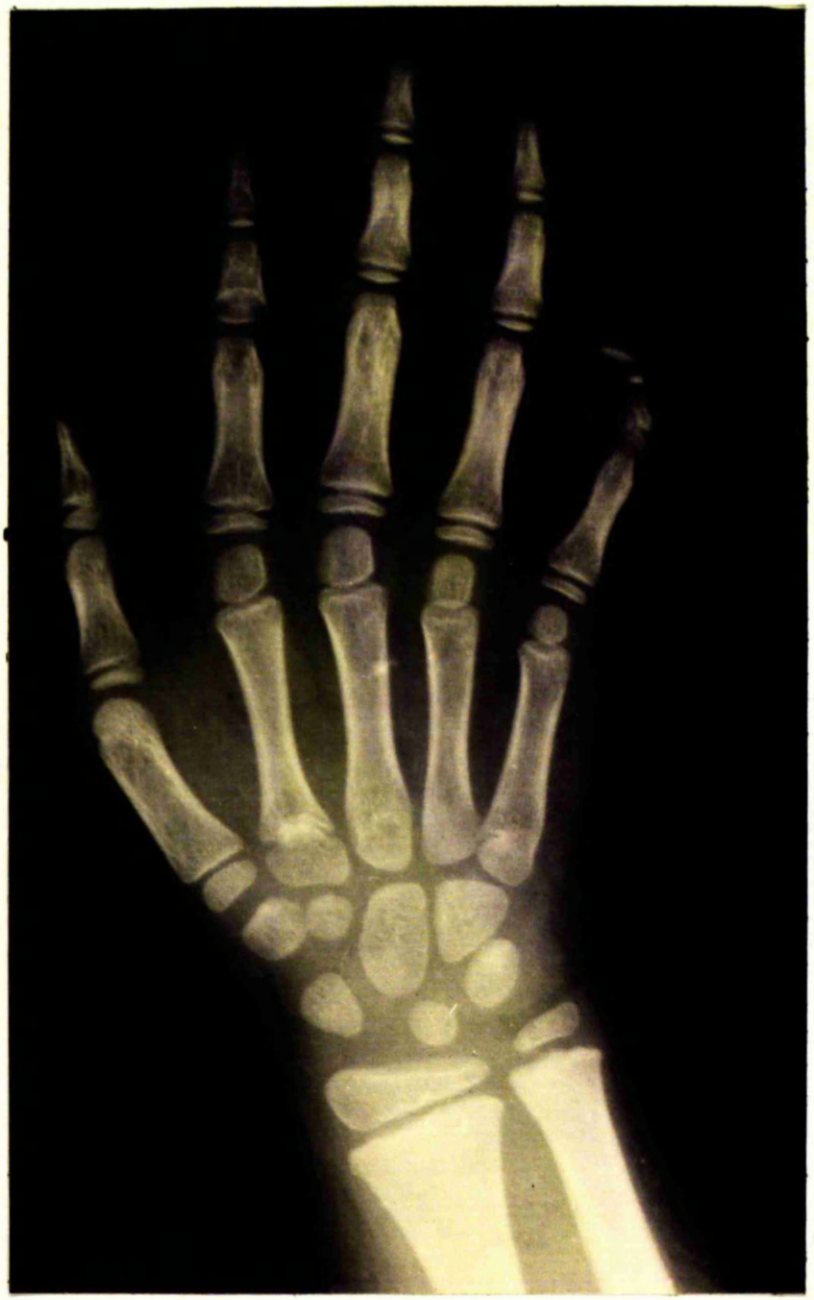

Figure 14 - X-ray of right hand showing an abnormal centre of ossification at the base of the second and fifth metacarpals, together with anomalies in the middle and terminal phalanges of the fifth finger.

The vas deferens appeared normally developed on both sides. Histological examination of a biopsy specimen of the right testis shows that compared with a normal testis of an 8 year old boy, (Fig. 15), the tubules are scanty and lie in an oedematous loose fibrous connective tissue stroma (Fig. 16). The tubules are of normal size and vary little in diameter. Some of the walls are slightly thickened, and these tubules appear to be becoming hyalinised. No recognisable germ cells are present, and only Sertoli cells are identified. The epididymis is infantile with widely separated tubules.

Sex chromatin

The percentage of nuclei containing sex chromatin varied considerably in several buccal mucosal smears, and at first the question of mosaicism was considered. In the smears with the highest percentage of chromatin-positive cells, 36% of suitable nuclei contained 1 sex chromatin body, 30% of nuclei contained 2 sex chromatin bodies, and 9.5% of nuclei contained 3 sex chromatin bodies (Fig. 17). 24.5% of nuclei contained no sex chromatin. In the histological sections of the testis and appendix, 1, 2 and, very occasionally, 3 sex chromatin bodies were visible in nuclei.

TABLE 5. FEATURES OF TRIPLE - X CASES

Author	Case No.	Age	I.Q.	Height (cms.)	Pubis to Sole (cms.)	Ratio Upper to lower Segment.	Span (cms.)	Maternal Age	Paternal Age	% Sex Chromatin			Frequency of Drumsticks	Menses	Fertility	Histology of Ovaries	Other Features
										Single	Double	Total					
Jacobs et al. (1959)	1	35	?N	176	-	-	-	41	40	57	14	71	8/500 1'Double'	From 14 to 19 irregular	0	Follicles deficient.	Breasts underdeveloped. Extern genitalia infantile. Vagina small
Jacobs et al. (1960)	2	21	High Grade	152	79	0.9	158	-	-	41	48	89	11/500	From age 9 regular	0	-	Flexion deformity fingers.
Stewart & Sanderson, (1960)	3	35	70	168	84	1.0	164	42	42	38	14	52	-	N.	4 chromatin negative males	-	-
Fraser et al. (1960)	4	30	50	156	80	0.94	156	28	30	43	30	73	5/500	From age 20	1 chromatin negative XY male	-	Epileptic
Fraser et al. (1960)	5	39	58	171	84	1.1	170	35	-	50	25	75	6/500 1'Double'	Irregular	0	-	Epileptic
Fraser et al. (1960)	6	61	38	151	72	1.11	147	40+	40-50	35	33	68	8/500	N.	0	-	Epileptic
Fraser et al. (1960)	7	73	50	156	77	1.02	154	-	-	33	30	63	8/500 1'Double'	-	0	-	Epileptic
Educationally Subnormal children. Fraser (Unpublished)	8	16	69	166	-	-	161	-	-	49	20	69	1	N.	0	-	-
	9	13	71	-	-	-	-	-	-	41	20	69	-	-	0	-	-
de Carli et al.(1960)	10	19	70	167	-	-	-	-	-	49	8	57	-	'Frequent and abundant'	0	-	'Paramongol' with slanting eye External genitalia & breasts underdeveloped. Uterus hypopl Thyroid mod. enlarged. I^{131} u increased with delayed excret
Sandberg et al. (1960)	11	21	40	172	-	-	-	41	49	-	70	-	5%	N.	0	-	Mongoloid facies. Webbed neck Flat occiput.
Johnston et al. (1961)	12	14	46	166	89	0.86	166	29	33	44	40	84	6/132	N.	0	Scanty follicles. Undifferentiated stroma.	Small head. "Mongoloid" palms.
Johnston et al. (1961)	13	26	<20	163	85	0.91	166	17	33	38	50	88	6/123	Irregular	0	Scanty follicles.	-
Johnston et al. (1961)	14	39	31	162	86	1.12	159	18	-	41	37	78	6/115	N.	0	Normal	-
Jacobs (1961)+	15	52	M.D.	155	78	0.92	156	34	33	-	-	-	-	13 to 17 years only	0	-	-
Jacobs (1961)+	16	47	M.D.	147	-	-	145	40	46	-	-	-	-	N.	0	-	-
Jacobs (1961)+	17	18	?N	156	71	1.14	156	33	34	-	-	-	-	N.	0	-	-
Penrose (1961)+	18	18	63	156	99	0.98	160	38	37	54	8	62	"High incidence"	N. but irregular	0	-	-
Hamerton et al. (1962)	19	23	M.D.	147	-	-	-	21	27	22	10	32	2/374	2 only since 16	0	-	Epileptic
Ridler et al. (1963)	20	21	63	156	79	0.99	160	38	37	54	8	62	1 'Double'	Irregular	0	-	Breasts underdeveloped. Axill hair scanty.
Ridler et al. (1963)	21	19	70+	161	78	1.05	161	-	-	38	22	60	?	N.	0	-	Breasts underdeveloped. Axill hair scanty. Epileptic.
Close (1963)	22	37	N	171	-	-	-	28	31	-	15	-	-	N.	2 Chromatin negative XY males	-	Normal female
Close (1963)	23	42	N	171	-	-	-	41	47	-	23	-	-	N.	0	-	Normal female. Diagnosed as thyrotoxic age 2o years.

+ Cited by Johnston et al. (1961), as a personal communication.

Figure 15 - Section of a normal testis of an 8 year old boy.
(Haemalum and eosin. X 140).

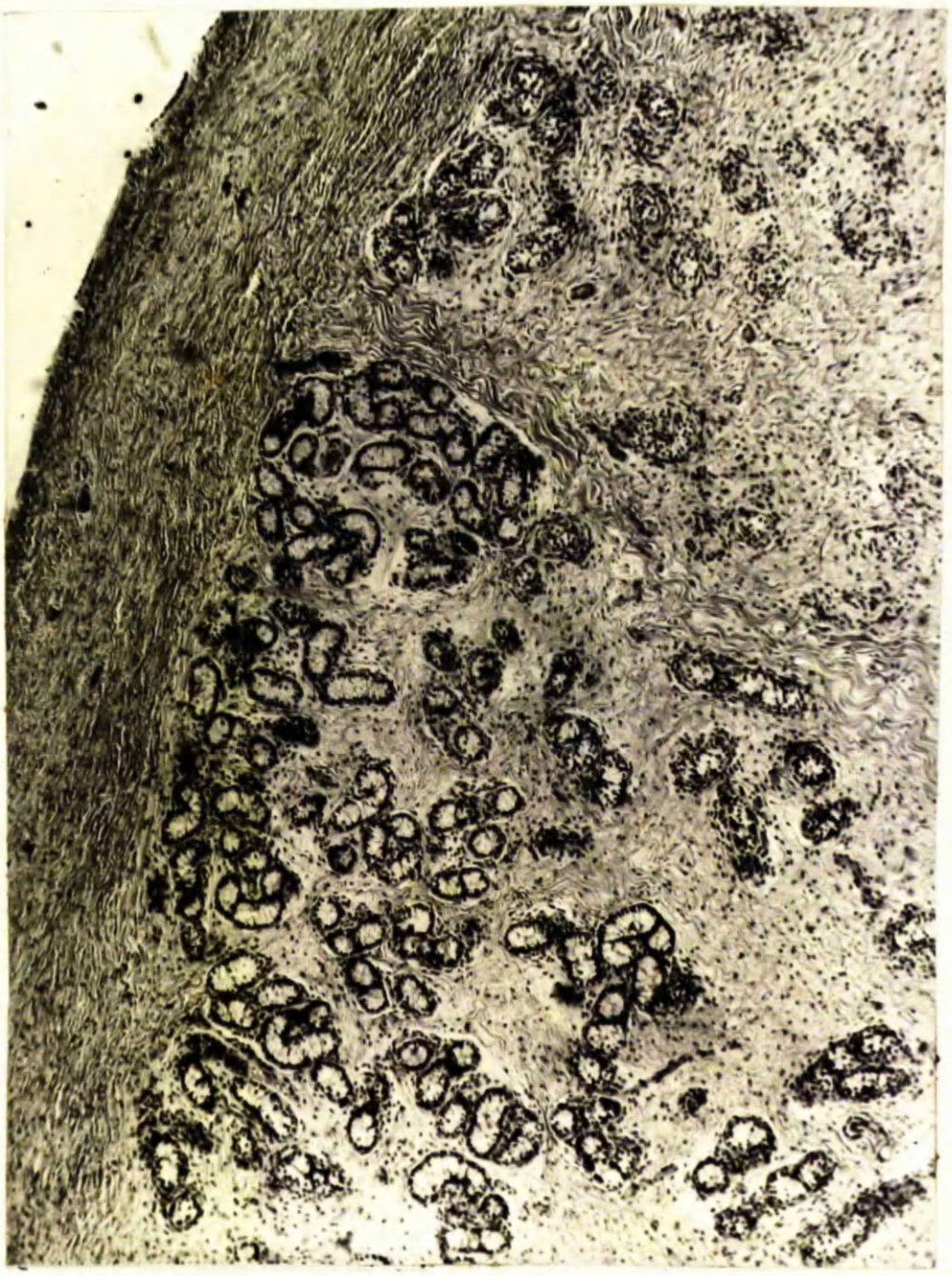

Figure 16 - Section of the testicular biopsy of the 8½ year old XXXXY subject, showing scanty tubules without germ cells lying in an abundant oedematous fibrous connective tissue stroma. (Haemalum and eosin. X 60).

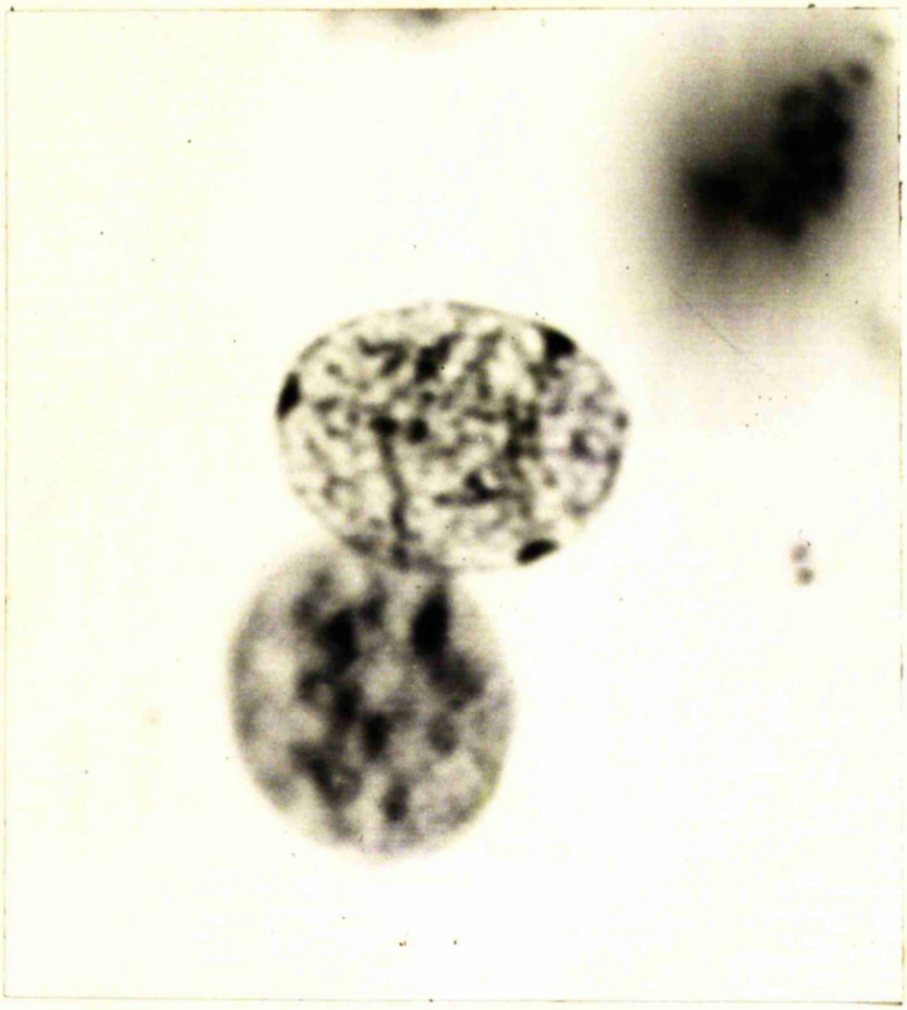

Figure 17 - Nucleus of oral mucosal cell containing three sex chromatin bodies. (Cresyl echt violet. X 2,500).

On examination of peripheral blood films, 6 drumsticks were found in 383 neutrophils. No neutrophil was observed containing two drumsticks.

Chromosome investigations

Mitotic chromosome counts were performed on cultures of peripheral blood, according to the method described by Moorhead et al. (1960). Over 90% of the cells counted contained 49 chromosomes. Of a total of 45 cells, 41 contained 49 chromosomes, 2 contained 48 chromosomes, and 2 contained 47 chromosomes.

Analysis of cells containing 49 chromosomes showed 22 pairs of autosomes together with a Y chromosome, and 4 extra chromosomes lying in the medium sized range (Fig. 18). On the basis of the sex chromatin findings, these were assumed to be X chromosomes. Tissue culture preparations from the abdominal skin, rectus sheath, peritoneum, testis and epididymis also showed a sex chromosome complex of XXXXY.

Comparison of patients with the XXXXY anomaly

In contrast to the XXXY chromosomal variant of chromatin-positive Klinefelter's syndrome, comparison of the

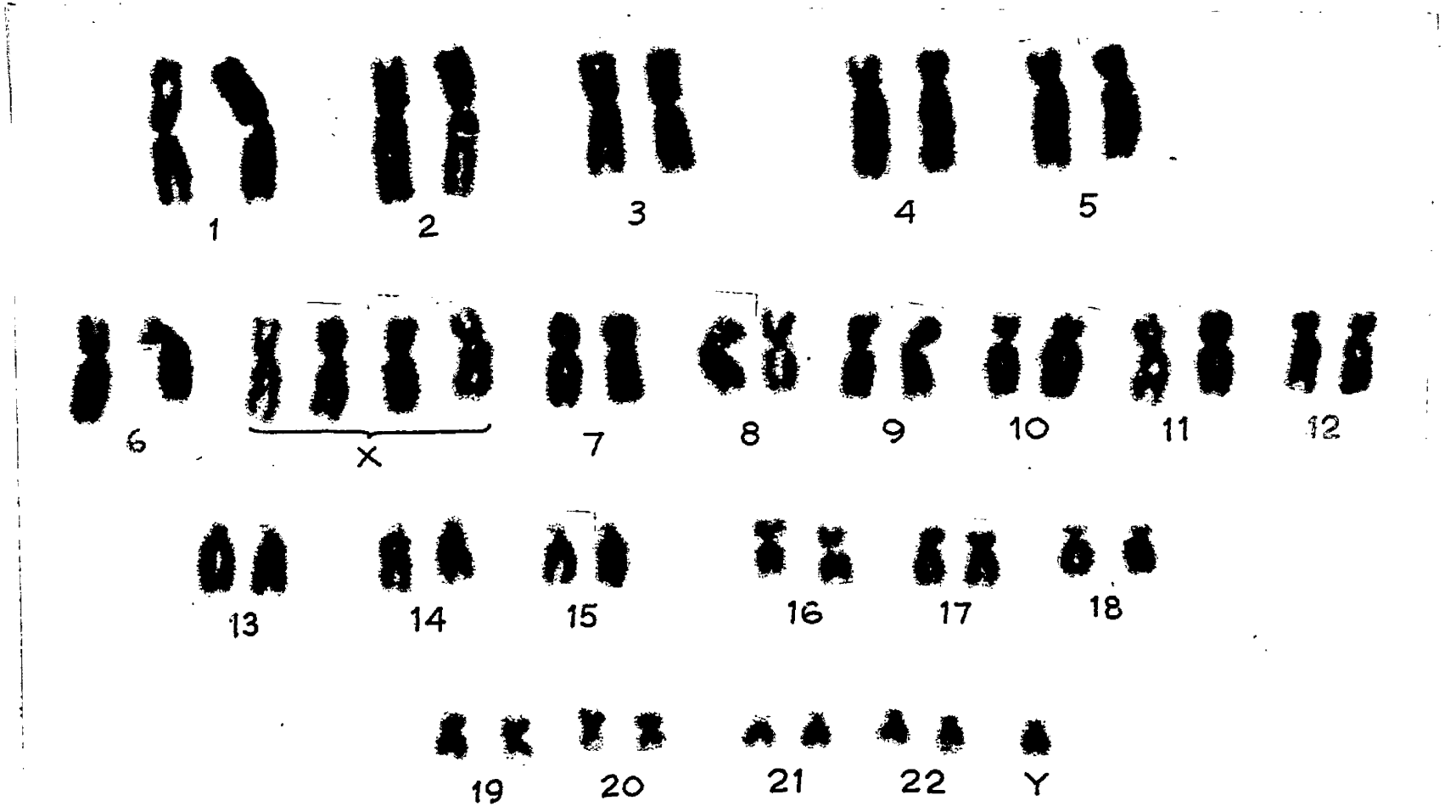

Figure 18 - Karyotype showing 22 pairs of autosomes together with the Y chromosome and the four presumptive X chromosomes, numbered according to the "Denver" system (1960).

ten cases with the XXXXY anomaly shows that several features are common to most of the cases in the group, which distinguish this variant from the usually encountered XXY form of the syndrome. Details of these cases are shown in Table 3, and analysis of the data reveals several interesting features.

With regard to the external genitalia, the scrotum is underdeveloped in all but one case, and in six of the ten cases the penis, also, is small. Bilateral undescended testes are present in five patients. In two patients, only one testis is descended, but in the remaining three cases, both testes are outside the inguinal rings. All the testes which are palpable are described as being small.

Biopsy of the testis has been performed in five children who were all under 9 years of age. In these cases, the testes showed varying degrees of abnormality. In the three older children, who were between 7 and 8½ years of age, there is a marked deficiency of seminiferous tubules in the testis, and no germ cells are present. In this age group, the most severe lesion is present in the case reported by Fraccaro et al. (1960c) in which the testis is described as being almost structureless, containing only a few tubules lined by Sertoli cells, together with other tubules obliterated by connective tissue. A

TABLE 3 XXXXY

KLINEFELTER'S SYNDROME

Author	Case No.	Age yrs.	I.Q.	Height (cms.)	Span (cms.)	Pubis to Sole (cms.)	Incidence of Drumsticks	Sex Chromatin Bodies % 1 2 3 4	Maternal Age	Paternal Age	External Appearances	Genitalia	Histology of Gonads	External Abnormalities	Other Features
Fraccaro et al. (1960) *	1	7	M.D.	-	-	-	'Present'	18 82 148 14 in 268 counted.	23	29	Flat occiput. Epicanthic eye folds. Protruding abdomen.	Scrotum and testes small.	R. testis - almost structureless. A few tubules containing Sertoli cells left in C.T. Other tubules obliterated by C.T. No germ cells or Leydig cells left.	Ossification centres of multangulum minus and capitulum radii absent.	Ductus arteriosus Enlarged heart. R. renal pelvis enlarged.
Anders et al. (1960)	2	8	21	-	-	-	6/600	52 21 9	29	29	Microcephaly. Esotropia. Myopia. Hypotonia.	Scrotum small. Testes undescended.	Hypoplastic tubules without spermatozoa.	Bilateral proximal radio-ulnar synostosis. Coxa Valga. Scoliosis. Pes planus. Mild pectus excavatum. Multiple pseudoepiphyses of hands and feet.	
Miller et al. (1961)	3	21	21	164	-	95	-	14%	23	32	Eunuchoid. Voice unbroken. Facial & axillary hair absent. Pubic hair scanty. Small stature. Strabismus. Cleft palate.	Penis and scrotum small. 1 cm. diam. mass L. side scrotum. Cord-like structure R. side scrotum.	-	'Multiple abnormalities'.	Maternal aunt and cousin are mongol Father died of leukaemia.
Fraser et al. (1961)	4	8½	33	107	95	46	6/383	36 30 9.5	22	24	Small stature. Cleft palate.	Scrotum small. Penis normal. Testes undescended.	Tubule scanty. No recognisable germ cells.	Sella turcica enlarged & deep. Bilateral coxa valga. Ulnae slender. Prox. part L. ulna expanded. L. radio-ulnar synostosis. Curved little fingers. Extra centres of ossification in hands.	
Fraccaro et al. (1962) §	5	12	35	147	140	-	6/3021	61 72 28 1 2000 counted	26	29	Mild hypertelorism. Prognathos. Neck short. Legs thin. Muscle power normal. Bilateral intention tremor.	Penis small. Scrotum rudimentary. Testes undescended.	-	Fusion of cervical arches, C4, C5, C6. Bilateral radio-ulnar synostosis. Middle phalanx of both 5th fingers short & curved. Glabella large. Frontal sinuses underdeveloped. Skull sutures prematurely fused.	
Barr et al. (1962)	6	4¾	20	100	98	48	7/1000	24 40 30	25	24	Short stature. Hypertelorism. Flattened occiput. Prominent forehead. High arched palate. Umbilical hernia. Peg teeth. Small chest & head. Divergent strabismus.	Scrotum normal. Penis v. small. Testes undescended.	-	Bone age retarded. Skull small. 6 lumbar vertebrae. Deficient arch L6. Bilateral cubitus valgus. Curved 5th fingers and toes.	Convulsions and abnormal E.E.G.
Barr et al. (1962)	7	7½ months.	M.A. 4-5 mths.	66	-	-	-	41 31 15	38	38	Small head. Hypotonia. Mild hypertelorism.	Scrotum small. Penis v. small. Testes small.	Germ cells reduced in number. Diameters of cords & tubules at lower limit of normal range. Otherwise normal.	Small skull. Radial heads absent. Radial & ulnar shafts short & bowed outwards. Bone age retarded. Bilateral radio-ulnar synostosis.	E.E.G. normal.
Pfeiffer (1962)	8	9 months.	-	-	-	-	127/5000	23 11 3	20	23	Hypertelorism. Myopia. Internal strabismus. Hypotonia. Delayed development. Hyperextensibility of joints.	Scrotum small One testis undescended	-	Brachycephaly. Flat occiput. Flat sella turcica.	Dilated ureter enlarged calyx one kidney.
Turpin et al. (1962)	9	7 months.	M.A. 5-6 mths.	-	-	-	-	6 20 'some'	25	-	Microcephaly. Hypertelorism. Retrognathia. Short neck. Pterygium colli. Hypotonia. Delayed development. Root of nose incurved & flattened. Eyes low in position.	Scrotum small. Penis v. small. Testes small.	Tubules irregularly distributed but of normal size. No Leydig cells. Looks like prepuberal testis.	Bones slender. Bone age retarded. Upper end of ulna enlarged. Little fingers incurved. 1st metacarpal short. Anterior fontanelle widely open.	
Schade et al. (1963)	10	24	M.D.	178	-	-	6/560	41 16 6	28	27	Eunuchoid. No axillary, pubic or body hair. Thin. Weak musculature. Prognathos.	Scrotum and penis small. Testes undescended.	-	Bilateral radio-ulnar synostosis. Cranial vault thickened. Frontal and axillary sinuses underdeveloped Delayed ossification.	

* Fraccaro et al. 1960c

Fraccaro and Lindsten 1960

reduction in the number of germ cells was also noted by Barr et al. (1962) in the testis of a 7½ month old baby, but on the whole, from the descriptions given, the testicular appearances in this case and in the 7 month old case reported by Turpin et al. (1962) are less abnormal than in the older children.

Apart from the gonadal lesions, all the patients show various skeletal anomalies. In eight of the ten cases, these anomalies include a bony abnormality of the forearm, and in five of these patients, a radio-ulnar synostosis is present. Other skeletal anomalies include abnormalities of the vertebrae, skull and sella turcica, cleft palate, coxa valga, retarded bone age and anomalies in the centres of ossification in the hands.

In many of the cases, the external appearance of the patient is markedly abnormal, due to the many congenital abnormalities present which may include, for example, microcephaly, flat occiput, prognathos, hyperteleorism and webbed neck. In addition, other anomalies such as hypotonia, strabismus and myopia are described. One patient also has a patent ductus arteriosus, and in this case, and in another patient, minor malformations are present in the renal tracts.

In the case reported by Pfeiffer (1962) the mental

status is not mentioned, but all of the remaining cases are mentally defective, and in at least five of these cases, in whom the I.Q. is 35 or less, there is a severe degree of defect. Although, therefore, the number of cases concerned is again too small for statistical analysis, it would appear that on the whole, the degree of mental defect is more severe than in the usually encountered XXY form of chromatin-positive Klinefelter's syndrome.

Comparison of the ten cases with the XXXXY sex chromosome anomaly shows that the most common features of this variant of Klinefelter's syndrome are a severe degree of mental deficiency, skeletal abnormalities involving especially the forearms, underdeveloped scrotum and penis, unilateral or bilateral undescended testes, and other variable congenital abnormalities.

One additional point of note in these XXXXY cases is that although three sex chromatin bodies are reported in varying percentages in all patients, in none of the seven cases in whom blood films were examined were three drumsticks observed, and only Pfeiffer has found double drumsticks. Of the 5,000 neutrophils Pfeiffer examined, double drumsticks were found in two, double sessile nodules were found in one, and in one further neutrophil, a drumstick and a sessile nodule were present together.

The significance of double drumsticks will be discussed in part 6.

Origin of the XXXXY anomaly.

The origin of this particular anomaly includes four possibilities which are rather similar to those which have already been discussed with respect to the origin of the XXXY anomaly. For reasons previously noted, secondary non-disjunction occurring in either parent, or primary non-disjunction taking place in both parents, is unlikely.

The third possibility is that non-disjunction might occur in both the first and second meiotic divisions in the mother, producing an XXXX ovum, which, when fertilised by a Y sperm results in an XXXXY zygote.

The fourth possible mode of origin is by non-disjunction in either parent producing an XXY zygote, which then undergoes mitotic non-disjunction at the first cleavage division, resulting in two lines of cells, i.e. XXXXY and Y. The latter being non-viable is eliminated, thus leaving the XXXXY cell line.

Either of these latter two explanations could adequately account for the XXXXY anomaly.

Other chromosomal variants

Apart from the XXY, XXXY and XXXXY chromosomal variants of chromatin positive Klinefelter's syndrome, various mosaic chromosome complexes have been reported, including, for example, XXY/XX (Ford et al. 1959c), XXY/XY, (Baikie et al. 1961), XXXY/XXXXY (cited by Harnden and Jacobs 1961), Buckton et al, unpubl. and XXY/XXxY in which one of the X chromosomes was partially deleted (Crawfurd, 1961b).

In addition to these chromosome anomalies, other complexes have been reported in which extra Y chromosomes are present as well as extra X chromosomes, giving rise to the variants XXYY and XXXYY. One subject with the latter anomaly has been described by Bray et al. 1963, and a total of four cases with the XXYY complex have been reported by Muldal and Ockey (1960), Ellis et al. (1961), Carr et al. (1961b) and Vague et al. (1961).

These five cases are of interest in assessing whether the extra Y chromosome has produced any specific lesion apart from those caused by the extra X chromosome. In this respect, it is pertinent to mention an XYY subject reported by Sandberg et al. (1961) together with two XYY children described by Fraccaro et al. (1962a).

The four XXYY patients and the XXXYY patient all show features of Klinefelter's syndrome, and are mentally defective. Testicular histology is reported in the XXXYY case and in three of the XXYY cases. In two of the latter cases, reported by Vague et al. (1961), and Ellis et al. (1961), the appearances, at least from the descriptions given, resemble those of the XXY testis, although in the case reported by Ellis et al., an unusual feature is the absence of the head and body of the epididymis. In the remaining XXYY case, and the XXXYY case, the testicular histology is markedly abnormal; in the former case it may be partly due to regressive changes in a 52 year old subject (Carr et al. 1961b) and in the latter case, to the effect of the extra X chromosomes (Bray et al. 1963).

With respect to the XYY subjects, the two children reported by Fraccaro et al. (1962a) are also mentally defective, and in one of these children, aged 8 years, both testes are undescended. The XYY case reported by Sandberg et al., however, appears to be a normal fertile male.

The only other points of note are the unusually long legs in one of the XXYY subjects (Carr et al.), and the markedly increased height of 193 and 196 cms. respectively, in the XXXYY

subject and in another XXYY case (Ellis et al.), both of whom also show prognathism and other features suggestive of acromegaly.

On the whole, therefore, the four XXYY cases present no common features which might delineate a clinical variant distinct from the XXY form of the syndrome.

With respect to all these subjects with extra Y chromosomes, it is possible that in some instances, the extra Y chromosome might have contributed to the mental defect and to the few other additional anomalies. These features are not, however, sufficiently consistent for any firm conclusion to be drawn, notably because the case reported by Sandberg et al. was a normal fertile male.

Although the effects produced by extra Y chromosomes are not yet firmly established, the presence of extra X chromosomes in the male may produce numerous anomalies, which in at least two chromosomal variants, form fairly specific syndromes. It would appear that, on the whole, an increase in the number of X chromosomes increases the severity of gonadal change, the degree of mental deficiency, and the frequency of associated congenital abnormalities of skeleton and soft tissues.

The Infrequency of Intersex States in Children.

Some of the anomalies of sex chromatin and sex chromosomes which have been described in this and in the preceding parts of this thesis, may be found in babies or children. For example, as I have previously mentioned, intersex states may be present at birth in children with ambiguous external genitalia. In such cases, as previously noted, nuclear sexing is of value in determining the sex in which the child should be reared, and also in recognising the important condition of congenital adrenal virilism, which if not diagnosed, may be fatal.

In addition, certain sex chromosome anomalies have been recognised in children, including, for instance, the XXXXY variant of Klinefelter's syndrome, in which, it may be noted, eight of the ten reported cases were identified in children under the age of 12 years. Two of the three cases with the XYY chromosome anomaly were also recognised in children.

However, it is important not to exaggerate the frequency of these intersex conditions in ordinary paediatric practice. For instance, working in the Pathology Department of the Royal Hospital for Sick Children in Glasgow for one year, I made, during this period, a careful search for nuclear

sex and sex chromosome abnormalities in children in the Medical and Surgical wards. These included females presenting with unilateral or bilateral inguinal hernias, or with webbed necks (possibly Turner's or Klippel-Feil syndromes); males with hypospadias or undescended testes, and one child with ambiguous external genitalia. During this year, in a hospital of 286 beds, only two anomalies were found. These were a female pseudohermaphrodite, and the XXXXY variant of chromatin-positive Klinefelter's syndrome described above.

PART 5

THE TRIPLE-X SYNDROME

The Triple-X Syndrome

In the previous two parts of this thesis, I have described how the application of nuclear sexing techniques to the study of subjects with anomalous sex development has led to the recognition of various sex chromosome abnormalities in both phenotypic males and females. The first two sex chromosome abnormalities to be described were the XO complex in chromatin-negative ovarian dysgenesis (Turner's syndrome), and the XXY complex in chromatin-positive Klinefelter's syndrome.

The third sex chromosome anomaly to be recognised was reported in 1959(b)by Jacobs and her colleagues, who described the clinical features of a phenotypic female who had the chromosome number of 47 and the sex chromosome constitution of XXX. This XXX chromosome complex is analogous to the XXX anomaly described by Bridges (1921) in the fruit fly, Drosophila melanogaster, in which the extra X chromosome gives rise to the so-called 'superfemale' fly. Jacobs and her colleagues therefore, at first, applied the same term 'superfemale' to this chromosome constitution in the human, but since then, the term has largely been abandoned in favour of the

purely descriptive term of 'triple-X'.

The first patient recognised as having this triple-X condition was investigated because of the symptom of secondary amenorrhoea. Clinical examination revealed various abnormalities of the reproductive system. The external genitalia were infantile, the vagina small, and the breasts underdeveloped. The urinary excretion of gonadotrophins was high. Biopsy of the ovaries, which resembled those of post-menopausal women, showed that although the patient was only 28 years of age at that time, a solitary primordial follicle and a later follicle with granulosa cell layer were the sole evidence of functional activity. No corpora albicantes were present.

Examination of her buccal smear showed that instead of one sex chromatin body as is found in the normal XX female, a proportion of the nuclei contained two sex chromatin bodies. In addition, in peripheral blood films, one of the 500 neutrophils examined showed two drumstick projections.

This sex chromosome pattern of XXX in a phenotypic female appeared to be the counterpart of the XXY chromosome anomaly found in chromatin-positive Klinefelter's syndrome. It had already been shown, (e.g. by Ferguson-Smith et al. 1957),

that this latter syndrome was associated with mental deficiency, and, as I have previously mentioned, Ferguson-Smith, in 1958, reported a high incidence of subjects with this XXY chromosomal anomaly in male mental defectives. Therefore, although mental deficiency was not present in the triple-X case described by Jacobs and her colleagues, it seemed logical to institute a similar search among female mental defectives, for patients with the XXX chromosome anomaly.

Accordingly, I investigated as many as possible of the female mentally defective patients who were inmates of Lennox Castle Hospital, Stirlingshire. Since Jacobs and her colleagues had shown that the XXX sex chromosome condition could readily be recognised by the presence of two sex chromatin bodies in nuclei of the oral mucosa, I decided to carry out the survey by means of the buccal mucosal smear technique of nuclear sexing as detailed below.

Methods

Smears from the buccal mucosa were taken from each patient who was in the hospital at the time of the survey. These smears were then fixed in Papanicolaou's fixative, (equal parts of 95% ethyl alcohol and ether), and stained with cresyl

echt violet according to the original method described by Moore and Barr (1955a).

Where unsatisfactory, the smears were repeated, but for various reasons such as death of the patient, difficult behaviour, or absence 'on pass' from the hospital, satisfactory smears were not obtained from 42 of the 637 female inmates. From the remaining 595 patients, good smears were obtained. In each case I examined 100 suitable nuclei, and recorded the percentage of nuclei containing a sex chromatin body situated at the nuclear membrane.

Results

In the smears from four of the 595 patients, a proportion of nuclei were found to contain two sex chromatin bodies. These four patients were then investigated for any significant features which might constitute a syndrome, and the results of the clinical investigations are described later in this chapter.

Of the 591 patients in whom the smears contained only one sex chromatin body, the percentage of nuclei in each smear containing sex chromatin is demonstrated in the accompanying histogram (Fig. 19). The range of nuclei containing sex chromatin varies between 20 and 72%, the chromatin body being most frequently found in 36 to 40% of nuclei.

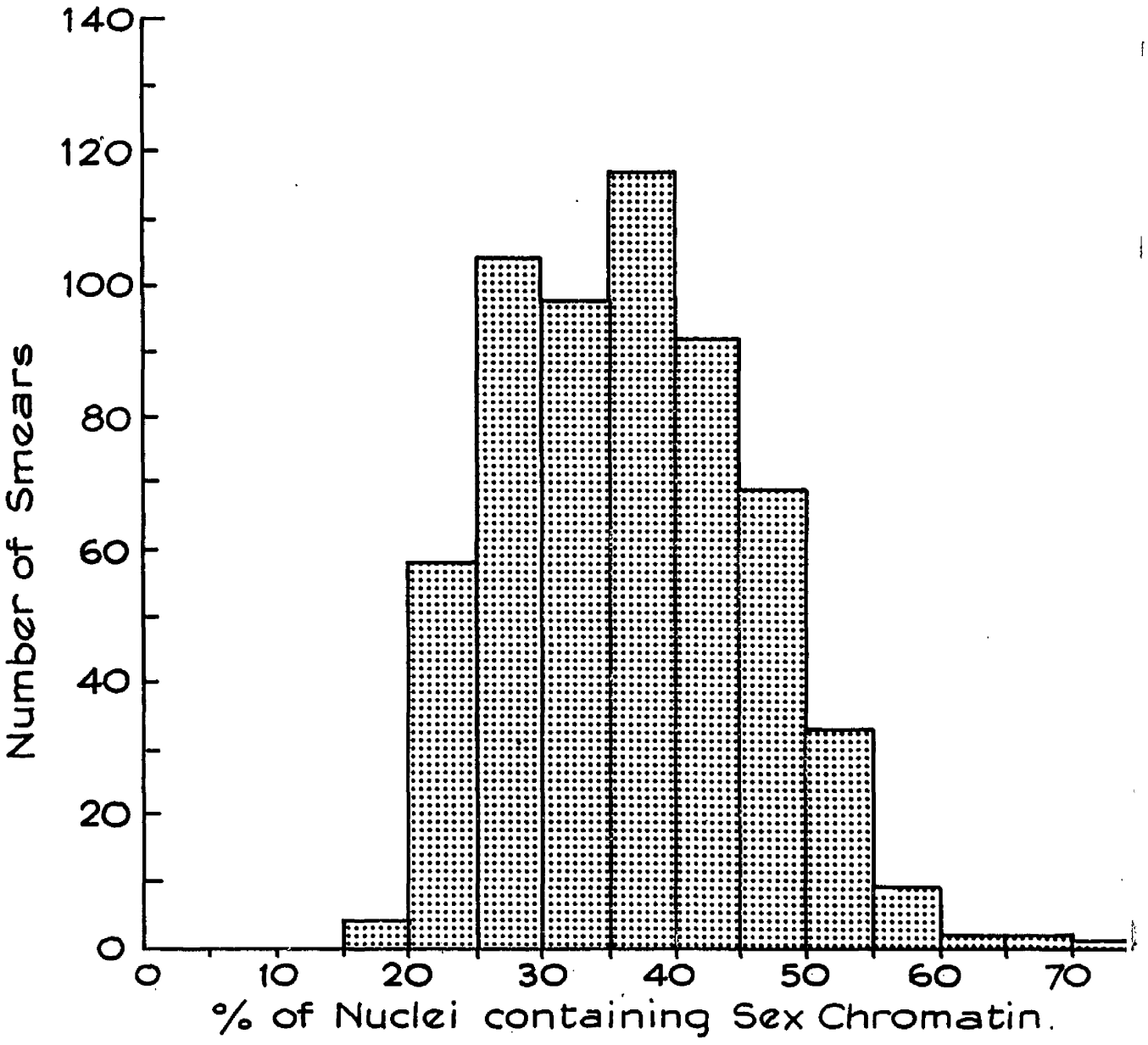

Figure 19 - Histogram showing the percentage of nuclei containing sex chromatin in the buccal smears of mentally defective patients at Lennox Castle Hospital.

Various figures have been reported for the percentage of nuclei containing sex chromatin in buccal mucosal smears. For example:-

Moore and Barr (1955a)	40 to 60%
Marberger et al. (1955)	20 to 79%, average 45%
Nelson (1956)	20 to 79%, average 53.5%
Maclean et al. (1961)	36 to 76%, average 49%
Ridler et al. (1963)	12 to 60%, average 33%

These findings show some degree of variation, but, as I have previously mentioned, several factors are involved including the quality of the preparation, the fixing and staining technique, and the criteria of the individual observer.

My own findings correspond most closely with those of Marberger et al. (1955) and Nelson (1956), although the figure of 36 to 40%, which in my survey is the most frequently encountered range of nuclei containing sex chromatin, is slightly lower than most of the averages given by other workers.

Sex Chromatin findings in the triple-X patients

With regard to the four cases in which two sex chromatin bodies were observed in some nuclei, the percentages

of nuclei containing sex chromatin are as follows.

Case No.	% nuclei with sex chromatin		
	single	double	total
1	43	30	73
2	50	25	75
3	35	33	68
4	33	30	63

From these figures it can be seen that the total percentage of nuclei containing sex chromatin in these cases, is higher than the average found in the remainder of the female population of the hospital.

As with the majority of workers, no unusual features were detected in the morphology of the double sex chromatin bodies (Fig. 20), which were almost invariably of equal size and were situated at varying positions around the nuclear membrane.

The frequency of drumsticks.

Blood films from each of the four patients were examined in order to estimate the frequency of drumstick projections in the neutrophils. The results were as follows.

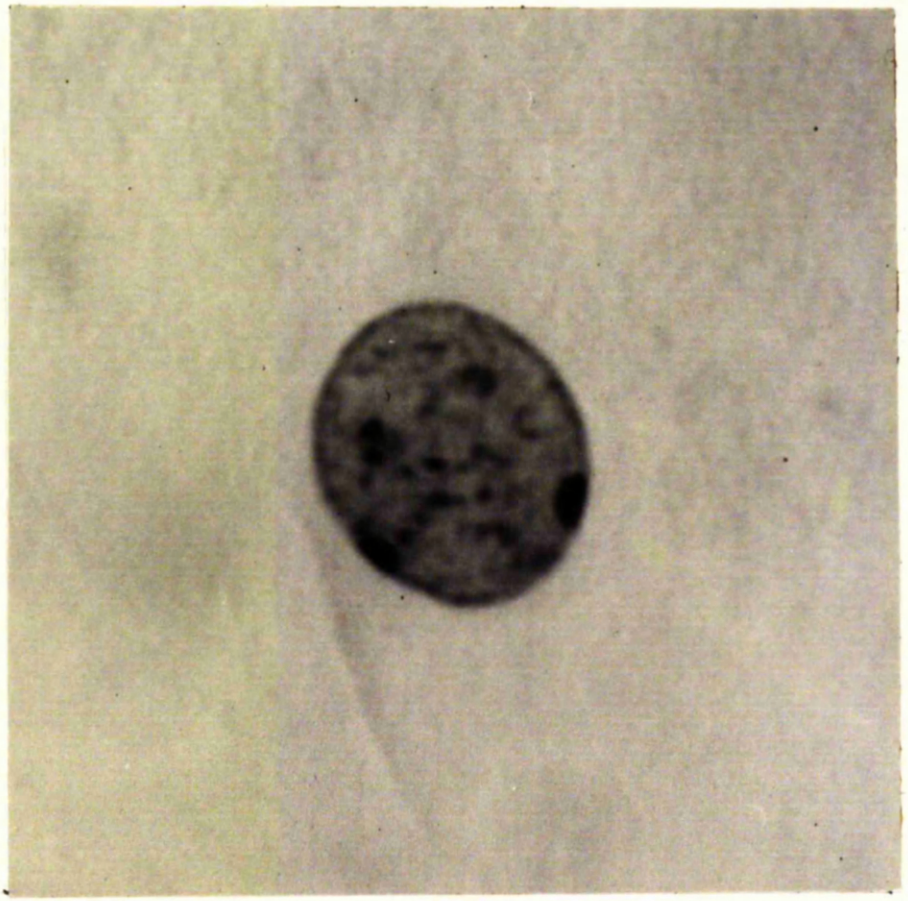

Figure 20 - Nucleus of an oral mucosal cell showing two sex chromatin bodies.
(Cresyl echt violet. X 2,500).

Case No.	No. single drumsticks	No. neutrophils examined
1	5	500
2	6	500
3	8	500
4	8	500

In contrast to the sex chromatin findings, the number of neutrophils with drumsticks is not greater than that found in the normal female.

In both case 2 and case 4, one neutrophil showed two projections from the nucleus which resembled drumsticks but were slightly more sessile in form (Fig. 21). This double projection is obviously analogous to the double sex chromatin body present in nuclei of the oral mucosa.

Chromosome analysis

Peripheral blood from cases 1, 2 and 4, was cultured according to the method of Hungerford et al. (1959), and sternal marrow from case 3 was prepared according to the method of Ford et al. (1958). The results were as follows.

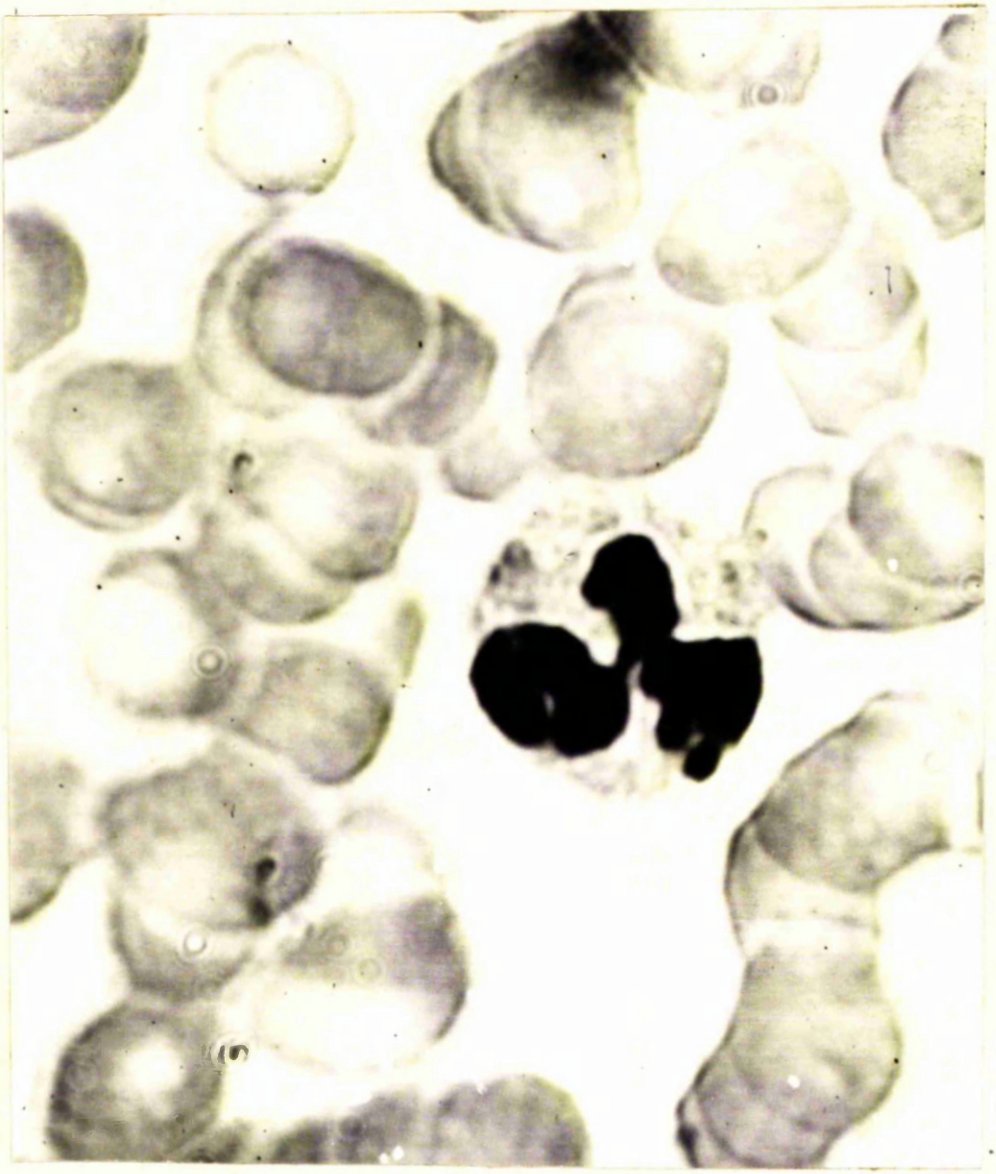

Figure 21 - Neutrophil showing two projections from the nucleus. (Leishman. X 3,000).

Case No.	No. of chromosomes		
	46	47	48
1	1	13	2
2	6	28	-
3	5	16	1
4	4	31	1

As can be seen, in each case there is a well-defined modal number of 47 chromosomes, although in cases 2, 3 and 4, the number of cells with 46 chromosomes is rather high. Analysis of the cells with 46 chromosomes, however, showed no consistent pattern, and analysis of those with 47 chromosomes showed that the extra chromosome lay in the medium sized range of chromosomes in which the X chromosome is found. On the basis of the sex chromatin findings, the extra chromosome in each case was assumed to be the X chromosome. The sex chromosome complex was therefore XXX (Fig. 22).

The four patients with this anomaly were then examined clinically in order to ascertain whether any features were present which might constitute a syndrome in the female in the same way as the XXY chromosome anomaly produces a specific syndrome in the male. The clinical details of the four patients are shown in table 4.

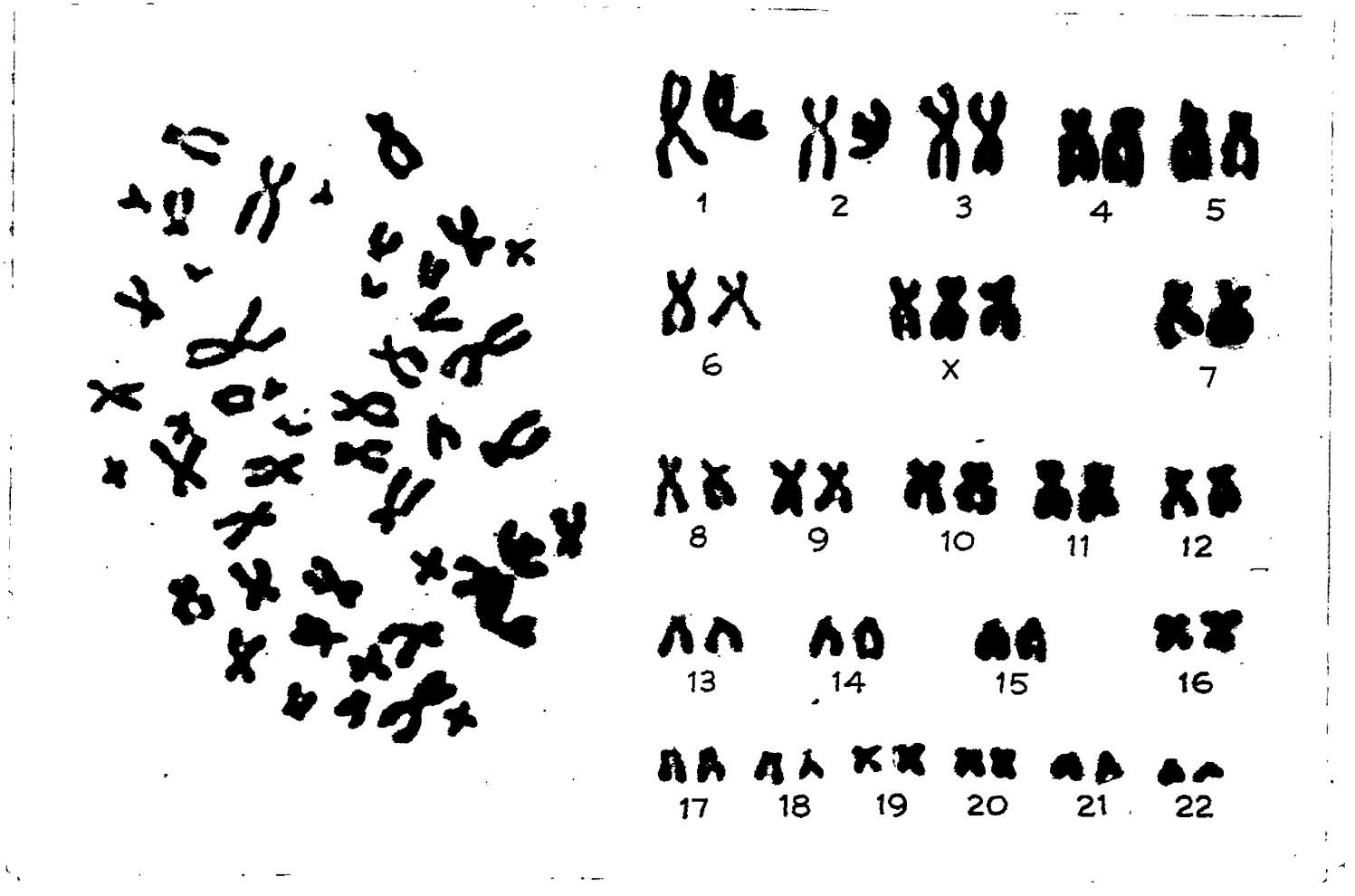

Figure 22 - Mitosis in a peripheral blood cell together with the karyotype showing 22 pairs of autosomes and the 3 presumptive X chromosomes, numbered according to the "Denver" system (1960).

Table 4
Features of Triple-X Cases

Survey	Case No.	Age	I.Q.	Height (cms.)	Span (cms.)	Pubis to Sole (cms.)	Menstruation: Age of Onset	Menstruation: Regularity	Fertility	Maternal Age	Paternal Age	Sibship Position	X-Ray Findings	Other Features
Lennox Castle Hospital	1	30	50	156	156	80	20	Regular	1 Male	28	30	4/8	Android Pelvis	-
	2	39	58	177	170	84	10	Irregular	0	35		5/5	Spondylosis. Calcified lesion apex R. lung ? Tuberculoma L. side skull	E.E.G. shows non-specific abnormality
	3	61	38	151	147	72	17-20	Regular	0	40+	40-50	10/11	Scoliosis and spondylosis. Osteitis frontalis interna	E.E.G.:- Parieto-temporal activity
	4	73	50	156	154	77	-	-	0	-	-	2/3	Spondylosis lower spine Opacity in R. occipital bone. Calcified nodule apex R. lung.	Positive W. R. Positive Kahn Test Hypertension
Educationally Subnormal Children	5	16	69	166	161	-	13	Regular	0	-	-	3/3	No abnormal features	-
	6	13	71	-	-	-	-	-	0	-	-	4/4	-	-

External Appearance

The external appearance of the four patients, whose ages vary between 30 and 73 years, is relatively unexceptional, (Figs. 23a,b,c,d) One of the patients who is 5 ft. 9¾ ins. (177 cms.) in height is rather tall, but the other three subjects are of average stature. Similarly, in only one case is the pubis to sole measurement greater than the head to pubis measurement; the other patients did not show increased length of the extremities such as is frequently found in subjects with XXY Klinefelter's syndrome.

Reproductive System

The secondary sex characteristics appeared normal in each patient. I was unable to obtain any details about the previous menstrual history of the oldest patient, but in the other cases, apart from the late menarche in two of them, the menstrual history was relatively normal.

Although the first XXX case described by Jacobs and her colleagues had markedly deficient follicle formation in the ovaries, one of the four patients found in my survey was proved to be fertile, for at the age of 27 years, this patient gave birth to a male child. Conception occurred while 'on pass'

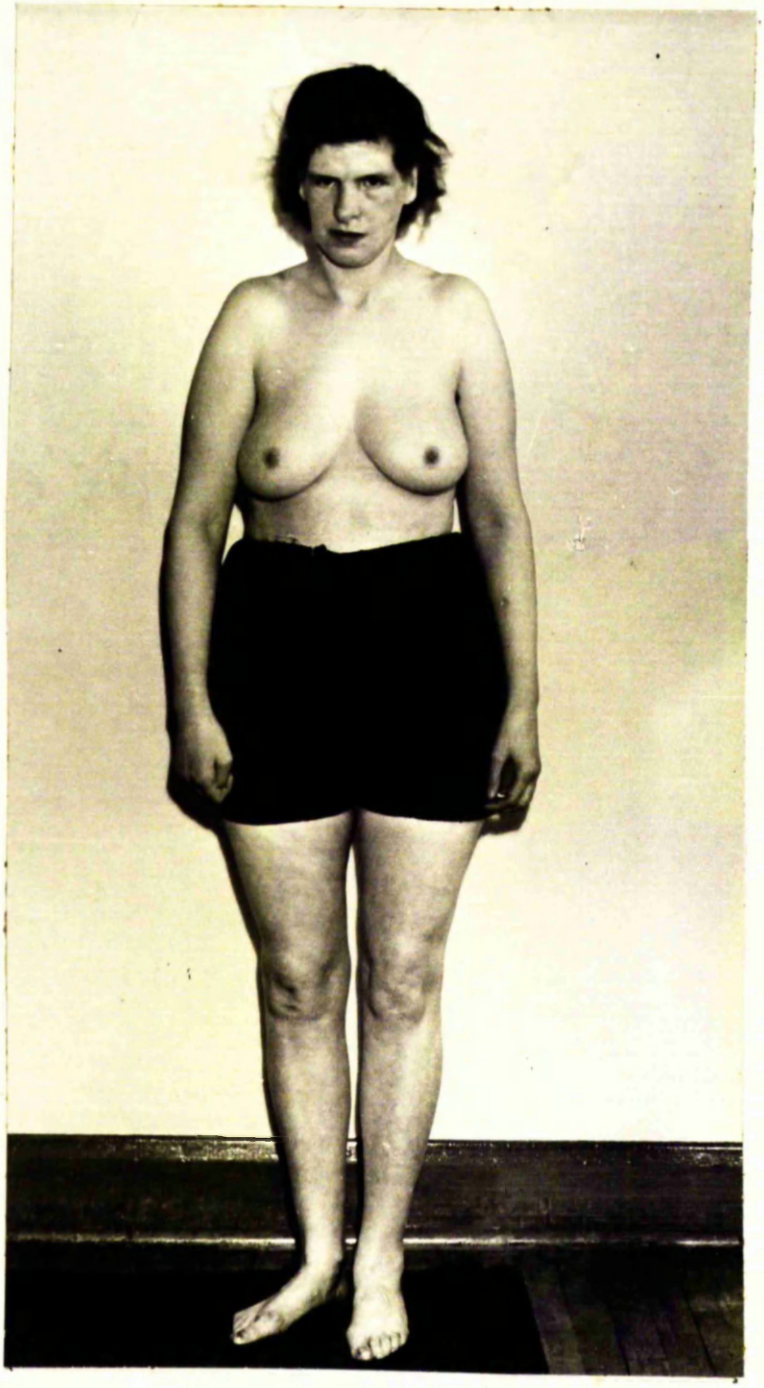

Figure 23a - External appearance of case No. 1.

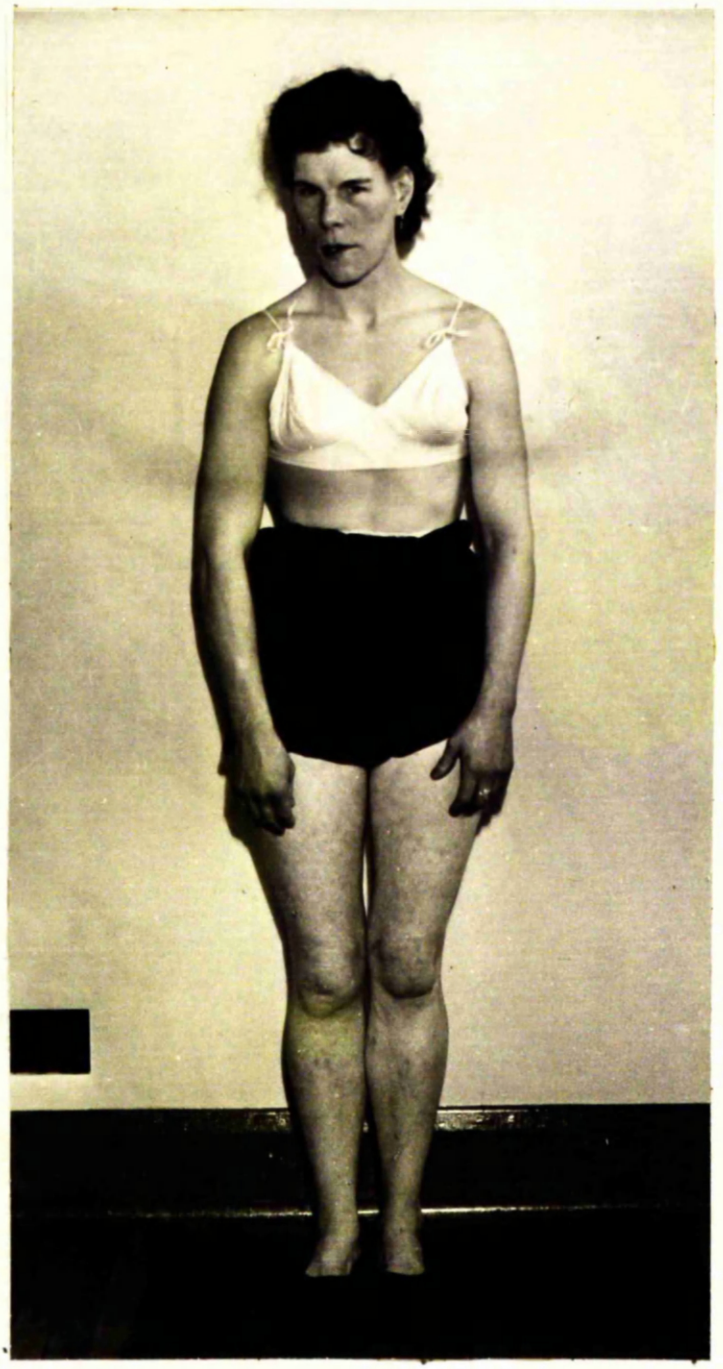

Figure 23b - External appearance of case No. 2.

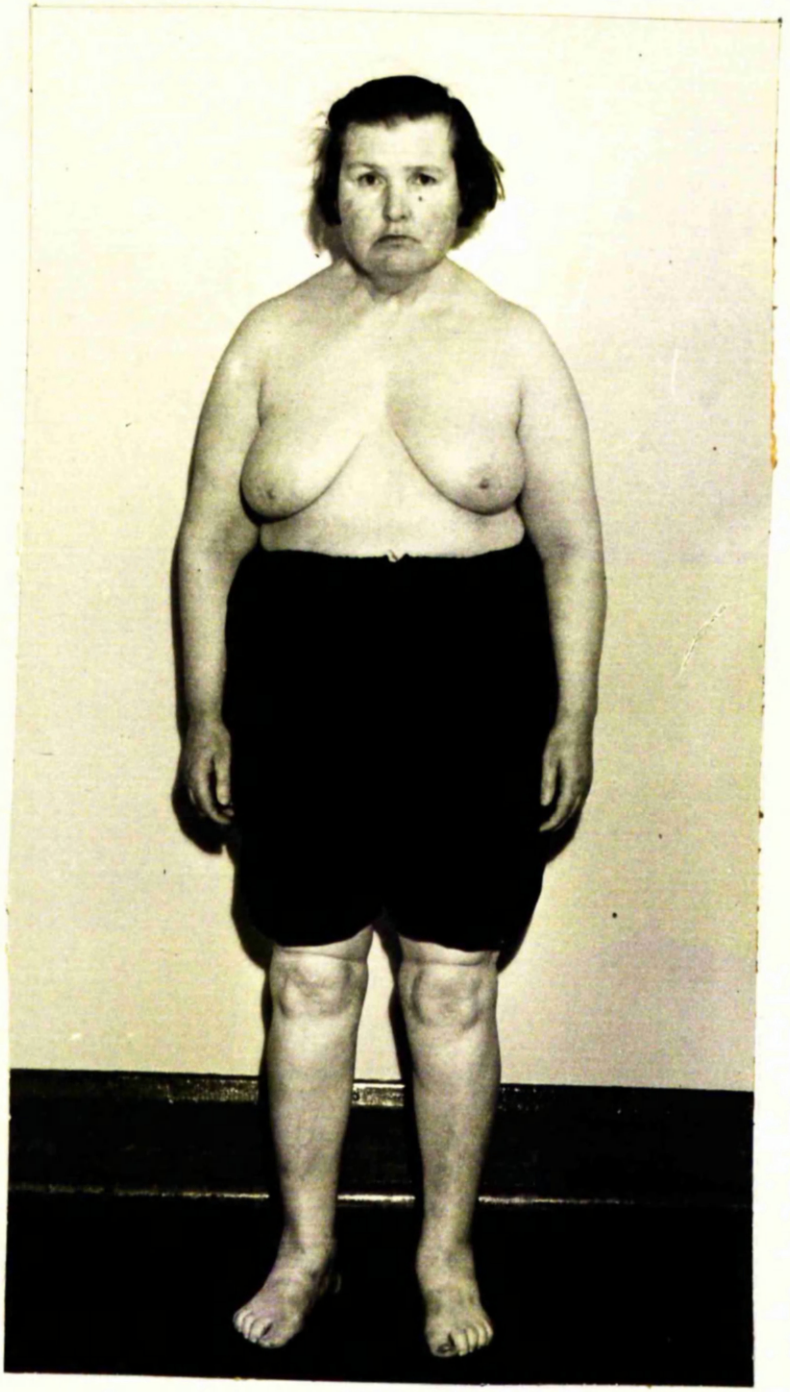

Figure 23c - External appearance of case No. 3.

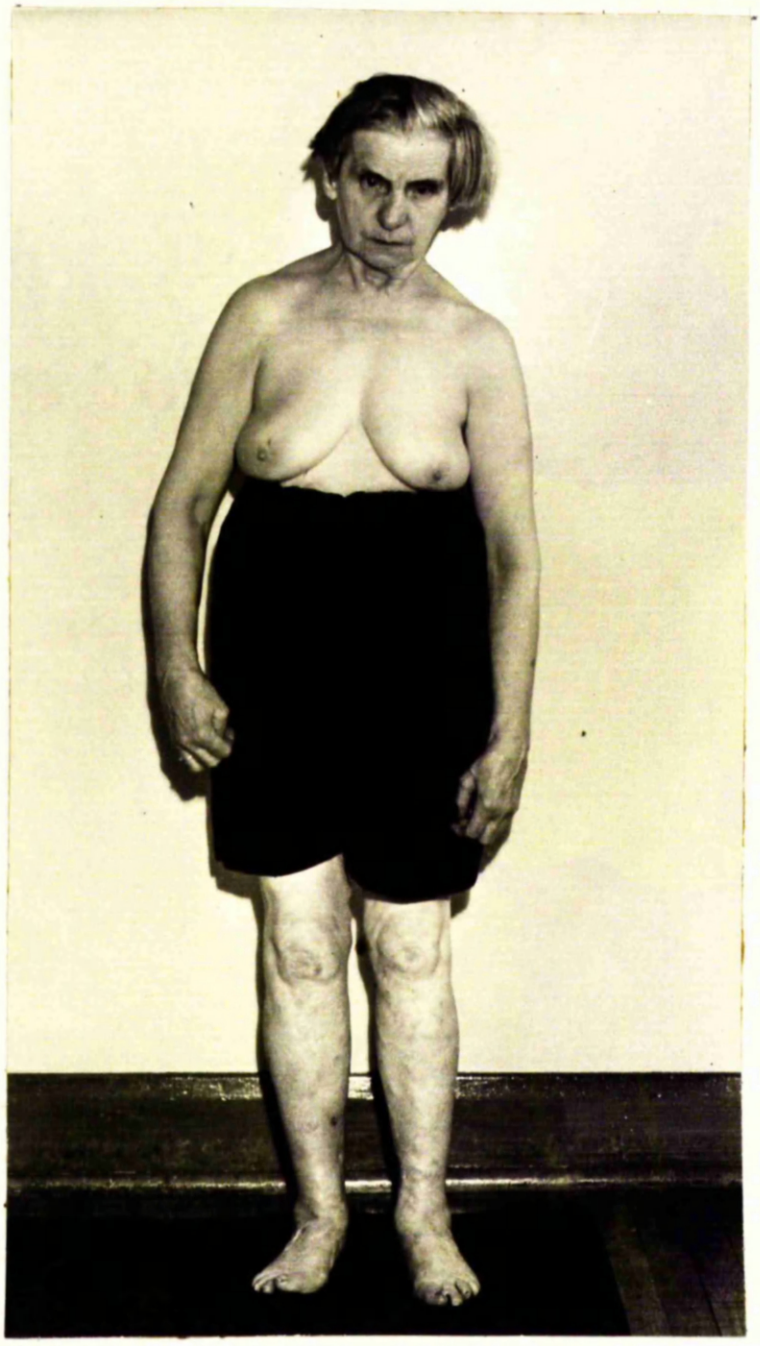

Figure 23d - External appearance of case No. 4.

from the hospital and the father is unknown. There is no doubt about the maternity, for the child was born in Lennox Castle Hospital, and then removed to an orphanage which I visited in order to make a physical examination and to obtain blood for chromosome studies together with oral mucosal smears.

The child at that time was 3 years of age, and no abnormality was revealed on physical examination. There appeared to be no evidence of mental deficiency and the staff of the orphanage had not noted any retardation of development. No sex chromatin was present in the child's buccal smears, and analysis of the chromosomes in peripheral blood cultures showed the number of 46, with the normal male sex chromosome pattern of XY.

Mental Deficiency

The I.Q. of the four XXX patients varies from 38 to 58. Two of the patients are imbeciles and two are feeble-minded. The type of mental defect is of the 'non-specific' variety, and there is no particular characteristic common to all which is of relevance in delineating a syndrome.

All four patients are epileptics, but this feature is probably not of any great significance since 25% of the

mental defectives in Lennox Castle Hospital are epileptics.

Other clinical findings

There are no striking clinical features common to the four patients, and many of the results of investigations are normal. Biochemical examination of the cerebro-spinal fluid and blood electrolyte levels shows no abnormality, and apart from confirming the presence of left ventricular hypertrophy in the oldest patient, who is hypertensive, the electrocardiograph findings are unexceptional. This latter patient who also has positive Wassermann and Kahn reactions, is almost blind and deaf, but the remaining three patients show no visual or ocular defects. Electroencephalographs show only some non-specific abnormality and parieto-temporal activity in two cases.

An X-ray examination was made of the whole skeleton in each of the four cases, but showed no evidence of the multiple bony abnormalities such as are found, for example, in XO chromatin-negative ovarian dysgenesis or XXXXY chromatin-positive Klinefelter's syndrome, and only a few minor features of no great significance were noted (see table 4).

Blood group studies show that all the patients are

group A, rh+ve, but this feature is probably coincidental.

Family Histories

Accompanied by a psychiatric social worker, I visited the nearest relatives of each of the triple X patients in order to obtain any relevant family histories, and also to obtain buccal smears.

Nothing of note emerged from the family histories except the high maternal age at conception of 35 and over 40 years of age in two cases. Buccal smears taken from the parents of case 1, a sister of case 2, two sisters of case 3, and a brother of case 4, were all found to show no abnormalities of sex chromatin.

Frequency of the XXX anomaly

In this survey, the incidence of the XXX sex chromosome anomaly was found to be 4 in 595 or 6.7 per thousand mentally defective patients.

However, owing to the policy of finding outside employment for as many patients as possible, relatively few of of the cases I examined at Lennox Castle Hospital were of the highest grades of mental defectives.

The less severe forms of mental defect are more likely to be found among mentally retarded children who are attending special schools for the educationally subnormal, and, as I have already mentioned in the previous chapter, various surveys of male children have been carried out by, e.g. Prader et al. (1958), Ferguson-Smith (1959), in order to determine whether the incidence of chromatin-positive Klinefelter's syndrome is greater in these higher grades of mental defect. In order to ascertain whether the incidence of the triple-X anomaly in female mentally defective children differed from the incidence in the female mental defectives at Lennox Castle Hospital, I therefore undertook a survey of all such children attending special schools in Glasgow.

The triple-X syndrome in mentally handicapped children

With the co-operation of the Education Department of Glasgow Corporation, I visited all the schools for mentally handicapped female children in the Glasgow area. From these 16 schools, I collected buccal mucosal smears from a total of 786 girls who were attending the schools at that time. 711 satisfactory preparations were obtained, and for this reason, repeat smears were not taken.

The method of fixing, staining and examining the smears was similar to that employed in my previous survey at Lennox Castle Hospital.

Results

In two of these 711 buccal smears, two sex chromatin bodies were found in a proportion of the nuclei. In the remaining 709 smears containing only one sex chromatin body, the percentage of nuclei containing sex chromatin is shown in fig. 24. The range of nuclei containing sex chromatin varies between 20 and 70% and the chromatin body is most frequently found in 36 to 40% of nuclei. These figures are almost identical with those which I obtained in my previous survey at Lennox Castle Hospital, (see p. 160)

The percentage of nuclei containing sex chromatin in the two triple-X cases is as follows:-

Case No.	% nuclei with sex chromatin		
	single	double	total
1	49	20	69
2	41	20	61

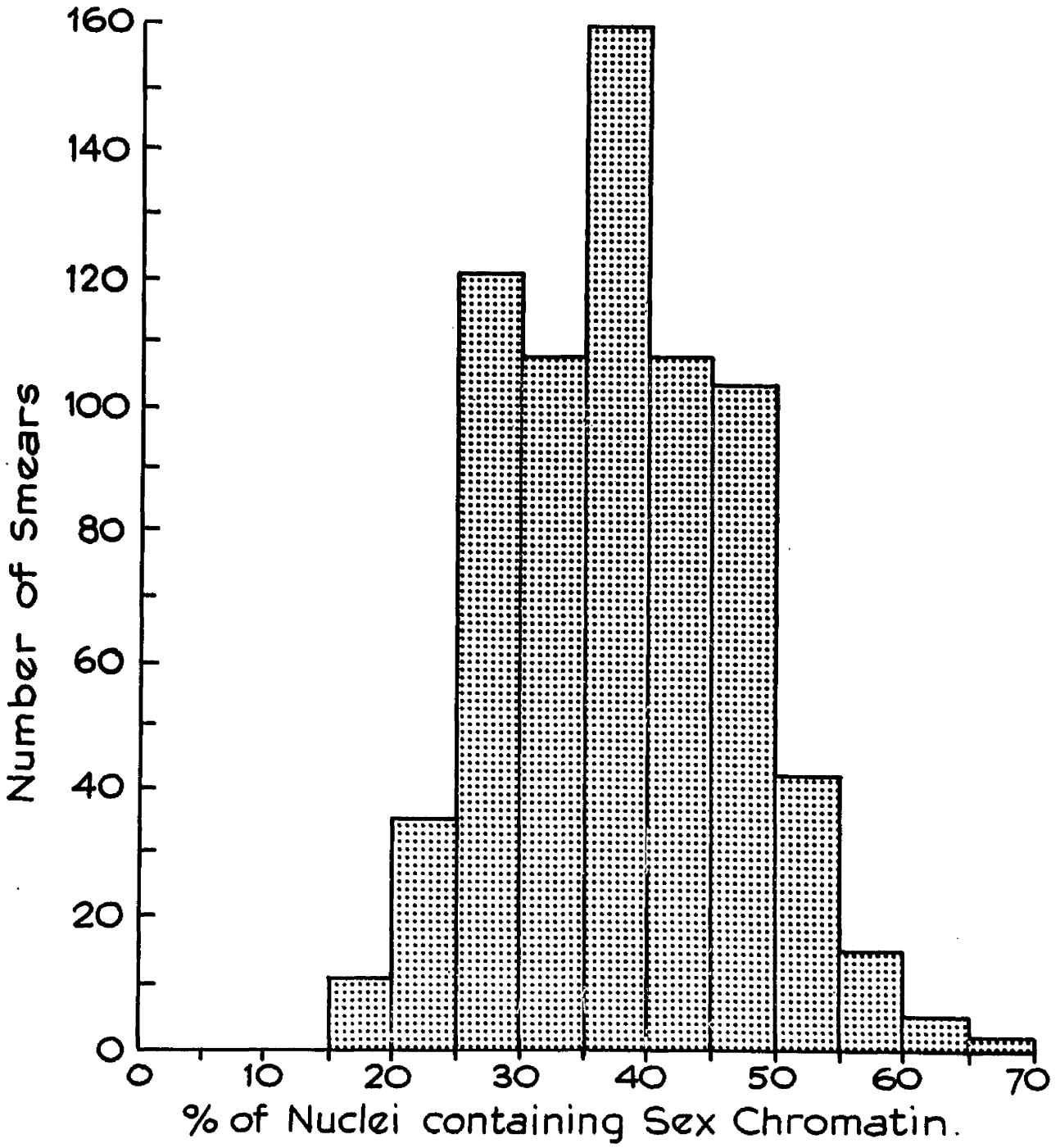

Figure 24 - Histogram showing the percentage of nuclei containing sex chromatin in the buccal smears of educationally subnormal children.

It is again to be noted that the total percentage of nuclei containing sex chromatin is higher than the average for the rest of the population surveyed.

Peripheral blood was obtained from case 1 and cultured according to the method of Hungerford et al., (1959). Of 39 cells counted, 35 had a chromosome number of 47, and 4 had a chromosome number of 46. Analysis of cells containing 47 chromosomes shows the sex chromosome complex of XXX.

Clinical features

Case 1, aged 16 years, has an I.Q. of 69. She is of average height (5ft. 5½ ins., or 166 cms.), and shows no evidence of increase in the length of the extremities. No abnormality was detected on physical examination, and an X-ray examination of the hands, feet and lumbar spine shows no significant features. Menarche occurred at the age of 13 years and menstruation is normal. She is the youngest of a sibship of three; her brother and sister are apparently normal. Case 2, aged 13 years

has an I.Q. of 71. She has two older brothers, together with a non-identical twin sister, who are all at schools for the mentally subnormal. No abnormality of sex chromatin is present in the buccal smear of the twin sister.

Unfortunately, the parents of this second case refused permission for further examination.

Comparison of patients with the triple-X anomaly

Comparison of patients with the triple-X anomaly

In addition to the original triple X case reported by Jacobs et al., (1959 b),and the four patients reported by my colleagues and myself (Fraser et al. 1960), descriptions of other patients with this sex chromosome anomaly have also been reported by Stewart and Sanderson (1960), Jacobs et al. (1960), de Carli et al. (1960), Sandberg et al. (1960a), Johnston et al. (1961), Hamerton et al. (1962), Ridler et al. (1963) and Close (1963); (see table 5). I have shown that there are no striking clinical features common to the triple-X patients found in my own surveys. Similarly, comparison of the clinical features of all reported triple-X cases also fails to reveal any specific syndrome.

Most of the patients present a fairly normal appearance. Analysis of the data shows no increased stature nor dis-

proportionate length of the extremities such as are found in XXY Klinefelter's syndrome. Neither, however, was there the shortness of stature found in XO ovarian dysgenesis.

The extra X chromosome in these patients does not appear to have a particularly marked effect on the secondary sex characteristics or on fertility. Abnormalities of secondary sex characteristics are present in only four of the 23 patients listed. In cases 1 and 10, the breasts and external genitalia are underdeveloped. The vagina in case 1 is small, and the uterus in case 10 is hypoplastic. In the other two patients (cases 20 and 21), the breasts are underdeveloped and axillary hair is scanty.

Secondary amenorrhoea is present in only three patients, (cases 1, 15 and 19), but of the remainder, the majority have a normal, or nearly normal, menstrual history.

Although, at first sight, the incidence of secondary amenorrhoea and of anomalies of secondary sex characteristics might appear to be rather high, it must be pointed out that patients with such abnormalities are particularly liable to undergo examination of buccal smears for abnormalities of sex chromatin. Consequently, the increased incidence of these anomalies in the reported triple-X patients may well be more

apparent than real.

Histology of the ovaries has been described in one patient, (as previously mentioned), by Jacobs et al. (1959b) and also in three other patients by Johnston et al. (1961). In one of these latter three cases, the ovaries present a normal appearance, but in the other two cases and in the case described by Jacobs et al., follicles are scanty.

Although abnormal appearances are present in the ovaries of three of the above four patients, one of my triple-X cases, (as previously described), was uniparous. Two other cases, one described by Stewart and Sanderson (1960) and the other by Close (1963), were also fertile and multiparous. Carr et al. (1961c) briefly mention a 60 year old mentally defective triple-X schizophrenic patient who has had nine pregnancies, and Lewis et al. (1963) also has reported a leukaemic patient who is a mosaic with the triple-X sex chromosome complex in one of her cell lines (XXX/XO), and who has given birth to three normal sons. Finally, Bergemann (1961a) has briefly and somewhat inadequately described a female mosaic with the triple-X cell line in the sex chromosome complex of XXX/XXXX. The mother and daughter of this subject also possess the same mosaic sex chromosome pattern. This unusual finding is rather difficult

to account for since such a familial mosaic abnormality would necessitate a somewhat similar process of maternal and zygotic non-disjunction in each case. This is a possible but rather unlikely phenomenon.

Apart from this latter case, of a total of 19 children born to triple-X mothers, the sex in 9 cases is not mentioned, but the remaining 10 children are described as normal boys, - a rather extraordinary fact for which no satisfactory explanation can be given. In 7 of these10 children, including my own case, nuclear sexing and/or chromosomal analysis has shown no abnormality. This point is of interest since, in theory, secondary non-disjunction in a triple-X mother should give rise to equal numbers of X and XX ova. Fertilisation of these would then give rise to XX, XY, XXX and XXY zygotes in a similar way to the reported cases of mothers with trisomy 21 or mongolism, (cited by Hamerton 1962), who by secondary non-disjunction have given birth to approximately equal numbers of mongol and normal children. However, as shown above, the XX, XXX and XXY complexes have not been found in the children of the triple-X mothers.

To account for the absence of sex chromosome abnormalities in these offspring, Polani (1962) has advanced two theories.

He suggests that either, for some reason such as preferential segregation, the abnormal gonia of these triple-X subjects are less able to mature into ova, or alternatively, that gonosomal mosaicism may be present, in which the gonads of triple-X patients might contain XX rather than triple-X cells. With regard to the former suggestion, it has been postulated by Ferguson-Smith (1964) that since oogonia do not contain a sex chromatin body, (i.e. do not undergo the process of 'Lyonisation' which will be discussed in part 6), sex chromosome abnormalities are more injurious to these cells than to other cells in the body. Consequently, oogonia containing an abnormal sex chromosome complex fail to mature. Although this hypothesis accounts for the absence of sex chromosome anomalies in the children of triple-X mothers, it does not, however, explain why these offspring are males.

Apart from the features mentioned above, the only other points worth noting in the clinical comparison of the triple-X patients are the rather questionable mongoloid features in three subjects, webbing of the neck in one subject, and the presence of epilepsy in six patients. Four of these, however, were found in my survey at Lennox Castle Hospital, and in these, as I have already noted, this feature is not considered to be of any great significance. In two patients, there was some

evidence of thyroid malfunction; one of the cases described by Close (1963) had been previously diagnosed as having thyrotoxicosis, and the patient reported by de Carli et al. (1960) had a moderately enlarged firm thyroid, with increased uptake and delayed excretion of I^{131}.

Comparison of the sex chromatin findings in the triple-X patients shows that, on the whole, the total percentage of nuclei containing sex chromatin is high, and similarly, in some of the cases, the incidence of drumsticks in neutrophils is slightly greater than normal. Double drumsticks have been noted in only four cases, and this point will be discussed in part 6 of this thesis.

The frequency of the triple-X syndrome

In part 4 of this thesis, I described the results of nuclear sexing surveys carried out by various workers on newborn male babies. Similar surveys have been carried out by these workers on female newborn infants, (table 6). Females with more than one sex chromatin body in their nuclei have been detected only in the surveys done by Maclean and his colleagues (1961, 1964). In 1961, these workers found four triple-X subjects in 3,000 babies (1.3 per thousand) and in 1964, they

Table 6

Frequency of Triple X Syndrome in General Population.

Author	No. cases	No. of XXX cases	% incidence of XXX
Moore (1959)	1804	0	0
Bergemann (1961b)	1838	0	0
Subray and Prabhaker (1962)	1832	0	0
Wiesli (1962)	1466	0	0
Maclean et al. (1964)	10000	12	0.12
	16940	12	0·071

reported that this survey had been extended to a further 7,000 babies in whom they found 8 more triple-X subjects, giving a total of 12 triple-X cases in 10,000 babies (1.2 per thousand). Although the combined figures from all the surveys give an incidence of 0.71 per thousand, the consistently higher results of the Edinburgh group suggest that the frequency is probably greater.

The incidence of the triple-X syndrome among mental defectives is somewhat higher than that in the general population. As I have shown, the incidence in my survey at Lennox Castle Hospital is 6.7 per thousand, and following this survey (Fraser et al. 1960), the results of several others have been reported by various groups of workers (table 7). The majority of these surveys have been carried out on female patients in institutions for mental defectives, or children with a severe degree of mental deficiency. In addition to my survey of educationally subnormal schoolchildren, one other survey in this less severely retarded group has been published by Chapelle and Hortling (1960).

The frequency of the triple-X syndrome in all these surveys is lower than the figure of 6.7 per thousand found at Lennox Castle Hospital, so that combination of the results gives

Table 7.

Frequency of the Triple-X Syndrome in Mental Defectives

Author	Population Surveyed	No. cases	No. XXX cases	% incidence of XXX
Fraser et al. (1960)	Mental Institution	595	4	0.67
Hamerton et al. (1962)	'Low Grade' Children	196	1	0.51
Olmstead et al. (1960)	Mental Institution	461	0	0
Johnston et al. (1961)	Mental Institution	827	3	0.36
Sanderson & Stewart (1961)	Mental Institution	240	1	0.42
Gustavson & Åkesson (1961)	Mental Defectives in Institutions and General Population.	40	0	0
Maclean et al. (1962)	Mental Institution	1907	7	0.37
Davies (1963)	Mental Institution	250	0	0
Ridler et al. (1963)	'Low Grade' Children	112	0	0
	Adults all grades	623	2	0.32
		5251	18	0.34
Chapelle & Hortling (1960)	Educationally Subnormal Children	269	0	0
Present Survey	Educationally Subnormal Children	711	2	0.28
		980	2	0.204
	Overall Incidence	6231	20	0.32

an overall incidence in all grades of mental deficiency of 3.2 per thousand.

In my survey of educationally subnormal schoolchildren, the incidence of triple-X cases is 2.8 per thousand and Chapelle and Hortling (1960) in a similar survey, have found none in 269 children. Comparison of this combined incidence in mentally subnormal schoolchildren (2.04 per thousand) with the overall incidence in mental institutions (3.4 per thousand) does not indicate that the triple-X anomaly is more frequent in the less severe forms of mental defect.

However, it has been shown by Kidd et al. (1963) that some triple-X patients in mental institutions are not intellectually subnormal, and that an accompanying psychosis may be the most prominent feature. Indeed, it has become apparent that mental deficiency is not a necessary feature of the syndrome, for recently, two triple-X subjects with normal intelligence have been reported by Close (1963). These two patients are of great interest since they are both apparently normal females. One of these subjects who is fertile, was detected on routine cervical cytological examination of patients attending a family planning clinic. The other was detected on cytological examination of sputum carried out as part of an

investigation for loss of weight.

This demonstration that the extra X chromosome need affect neither intelligence nor fertility, indicates that the general population must contain a proportion of apparently normal females with the triple-X chromosome anomaly who will be detected only by chance. Surveys of the adult general population as opposed to the population at birth would obviously be of interest in determining the incidence of such chromosome anomalies.

Association of the triple-X anomaly with other chromosome abnormalities.

The triple-X sex chromosome anomaly has been reported to be associated with trisomy of autosome 18 in two cases, (Uchida and Bowman 1961, Uchida et al. 1962, Ricci and Borgatti, 1963).

Trisomy of autosomes in the 16 - 18 group occurring without other chromosome anomalies, has been reported in several infants in whom multiple severe congenital abnormalities were lethal within several months of birth (Edwards et al. 1960, Smith et al. 1960, Crawfurd, 1961a). These abnormalities included such features as congenital heart disease, spasticity with probable mental defect, micrognathia, low-set malformed ears,

anomalies of the eyes, small mouth, flexion deformities of the fingers, syndactyly of the toes and other abnormalities of the feet, neck webbing, and deformities of the chest and cranium.

In the two cases in which trisomy 18 was found together with the triple-X anomaly, some of these congenital abnormalities associated with trisomy of the 16 - 18 group were present. The ovaries in both cases were abnormal, since follicles were deficient, and clumps of cells said to resemble epithelial cells, were present in the ovarian stroma.

The triple-X sex chromosome anomaly has also been reported to be associated with trisomy 21 in one patient by Yunis et al. (1964), and in another patient who also had a retinoblastoma, (Day et al. 1963). The latter authors comment that whereas they estimate that the coincidence of triple-X and trisomy 21 is 1 in 500,000 births, the association of these two chromosome anomalies with a retinoblastoma will only occur by chance in 1 in 12.5 billion (sic.) births.

In passing, it may be noted that trisomy 21 has also been described in association with the sex chromosome anomaly of XXY. Trisomy 21 was recognised by Lejeune et al. (1959) and Jacobs et al. (1959c), to be the cause of the well known clinical condition of mongolism, or Down's syndrome, and in the

occasional case where trisomy 21 has been found together with the XXY anomaly in the same subject, the clinical features of both mongolism and Klinefelter's syndrome have been present, (Ford et al. 1959a, Hamerton et al. 1962). Mongolism has also been noted by Mosier et al. (1960), and Miller at al. (1961) to be present in other members of the same family as subjects with chromatin-positive Klinefelter's syndrome.

Both mongolism and chromatin-positive Klinefelter's syndrome are associated with advanced maternal age, and Hamerton (1962), in his review of mongolism, notes that it cannot be concluded that the coincidence of these two conditions in the same subject occurs with greater frequency than might be expected by chance at any given maternal age.

Origin of the triple-X anomaly

The triple-X chromosome anomaly may theoretically arise by three possible mechanisms.

The first of these is the occurrence of non-disjunction at either of the meiotic divisions in the mother or at the second meiotic division in the father, producing an XX gamete, which, when combined with an X gamete from the other parent will give rise to an XXX zygote. However, as will be shown later,

the increased age of most of the mothers of triple-X subjects tends to support maternal as opposed to paternal non-disjunction.

The second possible mode of origin of the triple-X anomaly is the occurrence of secondary non-disjunction in parents who already have an abnormal chromosome constitution such as XXY or XXX. However, as previously mentioned, it has been difficult to prove fertility in an XXY male, and, apart from an XXX/XXXX mosaic briefly described by Bergemann (1961a), whose mother and daughter have the same sex chromosomal complex, all the parents and children of triple-X subjects who have been examined to date have shown a normal sex chromatin pattern. It is unlikely therefore, that secondary non-disjunction is the cause of the anomaly.

Finally, the demonstration that some subjects may have an XXX/XO mosaic sex chromosome complex (Jacobs et al. 1960), suggests that it is also theoretically possible for the XXX anomaly to arise from an XXX/XO mosaic zygote in which the XO cell line is eliminated. This possibility would be difficult to disprove, but the maternal age effect suggests that the underlying mechanism of the triple-X anomaly lies at the gametic rather than the zygotic level.

Effect of Maternal age on Non-disjunction

This is a convenient point at which to consider the effect of maternal age on non-disjunction.

Of the clinical conditions now known to be caused by a chromosome anomaly, the first to be studied with regard to the effect of maternal age was mongolism (or Down's syndrome). According to Hamerton (1962) it has been found that the frequency of mongol births is independent of the age of the father or the birth order, but increases with the age of the mother, and is greatest over the age of 40 years. This increased incidence of mongolism must therefore be related to the age of the oocyte influencing, in some way, the frequency of non-disjunction.

In the male, there is a rapid turnover of primary spermatocytes in the seminiferous tubules of the testis, with consequently little time for ageing processes to occur. In the female, however, as previously mentioned in part 2 of this thesis, it has been shown that, in the embryo, oocytes complete the entire process of the first meiotic prophase several weeks before the end of gestation, and then remain in a long interphase-like stage until shortly before ovulation, when meiosis is resumed. As Hamerton (1962) therefore remarks, "Some oocytes

remain in the ovary in a pachytene or post-pachytene stage for forty to forty-five years, and it seems not unreasonable to suppose that ageing might have affected them."

In addition to mongolism, a maternal age effect has also been noted in some of the sex chromosome anomalies.

For example, in my cases of the triple-X syndrome, a high maternal age of 35 and over 40 years is present in two of the three cases in which the age of the mother is known. Similarly, in the majority of the remaining 14 cases of the triple-X syndrome in which the maternal age has been reported, (table 5 p. 183) the latter is high, and of this total of 17 patients, the mean maternal age is 33.2 years. This is greater than the average maternal age of 28.5 years for the normal population in 1939 cited by Polani in his review (1962), and supports the theory that maternal non-disjunction is the cause of the triple-X anomaly.

According to Polani, whereas the majority of triple-X females are born at advanced maternal age (in this respect similar to mongolism), the maternal age effect for chromatin-positive males is less marked. Ferguson-Smith et al. (1964) have demonstrated that in cases of XXY Klinefelter's syndrome, the maternal age is high in proven cases of maternal non-dis-

junction but is normal when paternal non-disjunction is shown to be the cause of the anomaly. Consequently, as these authors point out, the maternal age effect for chromatin-postitive males is likely to be less marked than in mongolism because the contribution of cases due to paternal non-disjunction is greater in Klinefelter's syndrome. It may be noted, however, that the average maternal age of 25.9 years for the ten XXXXY subjects would suggest no maternal age effect at all in this particular variant of the syndrome.

Polani also states that the majority of XO and sex chromosome mosaic females are born at the average maternal age, and a few at advanced maternal age, and makes the interesting suggestion that the similar age effect in these two latter conditions might indicate a similar underlying mechanism acting at the zygotic rather than the gametic level. However, it may be observed that in the XO females, the lack of clear parental age effect supports also paternal non-disjunction as an alternative cause of the anomaly (Ferguson-Smith et al. 1964).

The effect of extra X chromosomes

In the male, the presence of one extra X chromosome produces specific lesions making up the well known clinical

features of chromatin-positive Klinefelter's syndrome. When more than one extra X chromosome is present, the extra chromosomes at least in the XXXXY chromosomal variant, are associated with the presence of additional congenital abnormalities such as skeletal malformations, together with an apparent increase in the degree of severity of the mental defect, and more marked abnormalities in the gonads and external genitalia.

In the female, as I have shown, extra X chromosomes are extremely well tolerated. In contrast to the male with the XXY sex chromosome complex, the extra X chromosome in the triple-X female produces few abnormalities. Mental deficiency, although present in the majority of cases so far described, is not a necessary accompaniment and in some subjects, even fertility is not affected. Indeed, the finding of the XXX chromosome complex in apparently normal females demonstrates how little effect may be produced, in some cases, by the presence of one extra X chromosome.

Two extra X chromosomes also appear to be well tolerated by the female, for the XXXX sex chromosome complex has been reported in two subjects with normal menstrual histories, in whom apart from severe intellectual subnormality, there were no physical anomalies (Carr et al. 1961a).

It has been shown, therefore, that both the male and the female tolerate the presence of extra X chromosomes remarkably well, and it is also of note, that in the male, a Y chromosome can produce a testis in the presence of up to four X chromosomes, even although the gonad formed is markedly abnormal.

The relatively small effect produced by an extra X chromosome is in marked contrast to the effect produced by the presence of an extra autosome. For example, trisomy 21 and trisomy of the 16 - 18 group, as previously mentioned, and also trisomy of the 13 - 15 group, (Patau et al. 1960), are all associated with multiple congenital abnormalities, which, at least in the latter two autosomal trisomies, may be sufficiently severe to be lethal within several months of birth.

This difference between the effect of extra X chromosomes and extra autosomes has been shown to be related to the nature of the sex chromatin body, and I now turn to consideration of the relationship between this structure and the X chromosome.

PART 6

THE DERIVATION OF SEX CHROMATIN

The Derivation of Sex Chromatin

One of the most interesting problems that has resulted from the application of nuclear sexing techniques to the intersex states concerns the nature of the precise relationship between sex chromatin and the X chromosomes. Over the last few years, this subject has evoked much controversy and speculation.

At the onset, Barr and his colleagues postulated that the sex chromatin body was derived from the heterochromatic regions of the two X chromosomes (Barr et al. 1950). This logical suggestion was supported by the occasionally observed bipartite appearance of the chromatin body, and each half of this structure was thought to represent one X chromosome.

This hypothesis was accepted for several years, although, even when introduced, there was raised the obvious objection that whereas, in the female, the two X chromosomes form a mass of approximately 1 μ diameter, the single X chromosome present in the male does not form a chromatin body of half this size, (Lennox, 1956b).

In recent years, however, several anomalies involving both the sex chromatin body and X chromosomes have been described, and in such cases, consideration of the numerical correlation

between the sex chromatin masses and the X chromosomes clearly shows that sex chromatin cannot be derived from two X chromosomes, as was originally thought.

For example, as I have already shown, (see parts 4 and 5 of this thesis), when a subject possesses three X chromosomes, as in the XXX and XXXY sex chromosome complexes, a proportion of the nuclei contain two sex chromatin bodies, and when four X chromosomes are present, as in the XXXXY complex, the nuclei may contain up to three sex chromatin bodies. If each sex chromatin body represented two X chromosomes, then in the former two examples, one would expect four instead of three X chromosomes to be present in the chromosome constitution of the subject, and in the latter example, where nuclei contain three sex chromatin bodies, one would expect six instead of four X chromosomes. Comparison of the number of sex chromatin bodies with the number of X chromosomes in these and other sex chromosome complexes shows that, with very rare exceptions, the maximum number of sex chromatin bodies which may be present in a nucleus equals one less than the total number of X chromosomes. This correlation, therefore, clearly demonstrates that sex chromatin can not be derived from two X chromosomes.

If sex chromatin is not formed from two X chromosomes,

then the obvious alternative is that it is derived from the heterochromatic region of only one X chromosome, and, indeed, several pieces of evidence have been advanced in favour of this theory.

In 1959, Ohno and his colleagues demonstrated that, in the rat, only one X chromosome in female somatic cells showed precocious heteropyknotic condensation in prophase. In 1961, Ohno and Makino, assuming logically that a similar situation might exist in man, extended their researches to the human sex chromosome field. Using foetal material, they showed clearly that in somatic cells in the human female, one X chromosome is precociously condensed at prophase. This observation strongly suggests that in intermitotic nuclei, only one X chromosome is heteropyknotic and may form sex chromatin.

The observation by Stewart and Sanderson (1961) of a sex chromatin body in 10% of germ cells in human testicular tissue, has also been advanced in support of the derivation of sex chromatin from a single X chromosome. However, although the description of this chromatin mass and the illustrations provided by these workers resemble those of the sex chromatin body found in the female, the fact that the XY bivalent in germ cells may be heteropyknotic necessitates a cautious inter-

pretation of their results.

Further information on the derivation of sex chromatin has been advanced by Grumbach and his colleagues, who, by meticulous observations, demonstrated the presence of sex chromatin in the XO cell line of a subject with ovarian dysgenesis whose sex chromosome constitution was composed of the mosiac complex XO/XX/XXX. (Grumbach et al. 1960, Grumbach and Morishima 1962). A further, rather similar case, has been reported by Jacobs et al. (1961); they described sex chromatin bodies which, however, were smaller than normal, in a subject with 45 chromosomes and the sex chromosome complex of XO.

Grumbach and his colleagues have maintained that their observations prove that the sex chromatin body must have been derived from the single X chromosome in the XO cell line; it is, however, difficult to reconcile the possibility of a viable chromatin-positive XO cell with the hypothesis that one X chromosome is "necessary for, and engaged in the metabolic business of the cell and therefore not stainable". This hypothesis will be discussed in due course. It is therefore possible that the explanation proposed by Jacobs et al. for their chromatin-positive XO case may also apply in the case described

by Grumbach and his colleagues, i.e. that there may be a small duplication or translocation of some of the X chromosome material onto an autosome, the abnormality being too small to be detected by present techniques.

Nevertheless, although the evidence advanced by Stewart and Sanderson, and Grumbach and his colleagues, may not be completely acceptable, the observations made by Ohno and his colleagues, together with the numerical correlation between sex chromatin and the X chromosomes, provide strong evidence that sex chromatin is derived from the heterochromatic region of one X chromosome.

There still remains to be accounted for, however, the bipartite appearance of the sex chromatin body, and two suggestions have been proposed. Firstly, Ohno et al. (1959) have postulated that this appearance may be due to the X chromosome being folded back on itself at the centromere. Secondly, Barr and Carr (1962a) have suggested that the structure may represent the heteropyknotic regions of the two chromatids of the X chromosomes. Either of these possibilities would adequately account for this phenomenon.

The derivation of drumsticks

In part 2 of this thesis, I discussed the relationship between drumsticks and sex chromatin, and indicated that it is now thought that the drumstick is merely an extruded sex chromatin nodule. Further observations by various groups of workers on morphological abnormalities in sex chromatin bodies, drumsticks and X chromosomes have given strong support to this hypothesis.

For example, as previously discussed in part 3, certain subjects with ovarian dysgenesis have been found to show variations in the size of their sex chromatin bodies. When the chromatin mass was larger than normal, one of the two X chromosomes was also found to be larger than normal, and was thought to represent an isochromosome for the long arm of the X (Fraccaro et al. 1960, Jacobs et al. 1961). When the sex chromatin body was smaller than normal, one of the two X chromosomes was also smaller than normal, due to deletion of either the short arm, (Jacobs et al. 1961), or the long arm of the X, (Jacobs et al. 1960).

It has also been demonstrated by Maclean (1962), that in similar circumstances, analogous morphological abnormalities may likewise be present in drumstick projections, for he has

shown that large drumsticks may also be associated with an isochromosome of the long arm of the X and that small drumsticks may be associatied with deletion of the small arm of the X.

This morphological correlation between the sex chromatin bodies and drumsticks in similar circumstances therefore strongly supports the view that the two structures are closely related and represent different forms of the same phenomenon.

As I have indicated, strong evidence has been provided in favour of the theory that sex chromatin is derived from a single X chromosome, and similarly, evidence has been advanced which suggests that the drumstick projections in neutrophils are also related to one X chromosome.

For example, Davidson and Winn (1961) have shown that the ratio of the drumstick head to the whole neutrophil nucleus is 1 to 34, and that the ratio of the mass of the X chromosome pair to all the chromosomes is 1 to 18. Although the technique used for this demonstration was admittedly rather crude, nevertheless, this striking difference in the two ratios strongly suggests that the drumstick projection also is formed from a single X chromosome.

Although, as previously mentioned, there are definite numerical correlations between sex chromatin bodies and X chromosomes which support the derivation of sex chromatin from a single X, similar correlations between drumsticks and X chromosomes are not, on the whole, so clearly demonstrated.

I mentioned, in part 5 of this thesis, that double drumstick projections analogous to double sex chromatin bodies were identified in two of my four triple-X cases identified at Lennox Castle Hospital. Double drumsticks were also described in the original triple-X case reported by Jacobs et al. in 1959(b); Maclean (1962) in a survey of subjects with abnormalities of X chromosomes has since confirmed the presence of double drumsticks or double sessile nodules in further triple-X subjects.

Although it may be observed that the drumstick findings in individuals with two and three X chromosomes correspond with the sex chromatin appearances, in no subject with four X chromosomes have three drumstick projections been identified in neutrophils. Such cases include the XXXXY males previously described in part 4 of this thesis, and the two XXXX females reported by Carr et al. (1961a).

A further point worthy of note is that although, as

may be observed from tables 2, 3, and 5, the presence of extra X chromosomes, on the whole, increases the incidence of the total number of nuclei containing sex chromatin, the frequency of drumstick nodules in neutrophils, in the majority of cases, is not increased.

As I have indicated, the numerical correlation between drumsticks and X chromosomes is not as clearly marked as between sex chromatin and X chromosomes. It is possible, as Maclean suggests, that the absence of three drumsticks in the presence of four X chromosomes may be due to the technical difficulties of identifying drumsticks which lie on top of the main nuclear mass of the neutrophil. It might also be postulated however, that the sex chromatin body, although condensed, is not yet extruded from the nucleus in the form of a drumstick or sessile nodule.

The nature of sex chromatin

Although it is now established that sex chromatin is derived from a single X chromosome, much speculation has been aroused in the last few years concerning its presence in the intermitotic nucleus. One of the more interesting problems that has arisen concerns the numerical correlation between sex

chromatin bodies and X chromosomes. As I have previously shown, the maximum number of sex chromatin bodies that may be present in a nucleus is one less than the number of X chromosomes. Several hypotheses have been advanced to account for this phenomenon.

One such view which was proposed in a leading article in the 'Lancet' (1960), suggested that one set of autosomes in a cell suppresses the heteropyknocity of one X chromosome. This theory is supported to some extent, by the work of Böök and Santesson (1961) on cultures of triploid XXY cells obtained from a boy with malformation syndrome. Sex chromatin bodies were observed in only a few cells of the several thousand screened.

As previously indicated, whereas an increase in the number of autosomes causes numerous congenital abnormalities which are often lethal, an increase in the number of X chromosomes appears to be remarkably well tolerated by the body. In the male, even three extra X chromosomes are compatible with life and, although modifying the structure and function of the testis, do not prevent the initiation of formation of this organ by the Y chromosome.

One of the best theories to account for these facts

was advanced by Stewart (1960) who states that "In the inter-mitotic metabolic nucleus, the heterochromatin of one X chromosome is apparently necessary for, and engaged in, the metabolic business of the cell and therefore not stainable. The heterochromatin of any other X chromosome is, however, superfluous to the metabolic requirements, functionally inert at this time, and therefore stainable, usually at the nuclear membrane."

Later, a leading article in the 'Lancet' (1962) very aptly expressed this hypothesis when it stated that "the two X chromosomes can exist in two different forms, one working like Cinderella in the background, while any others present in the cell behave like her ugly sisters, doing no work, but insisting on being seen".

This suggestion fits fairly well with the facts, and if combined with the previous hypothesis (see p. 212), proposed by the 'Lancet' (1960), also accounts for the sex chromatin findings in polyploid cells, since it may then be postulated that each extra autosome set will need an extra working X chromosome, (Lennox, 1961).

Strong evidence that only one X chromosome is fully active in the diploid cell is provided by Grumbach and his

colleagues (1962). These workers demonstrated that the activity of the enzyme glucose-6-phosphate dehydrogenase (G-6-P.D.) was within normal limits in 14 of 15 subjects in whom there was an abnormal number of X chromosomes. Since it is thought that an X-linked gene controls the activity of the enzyme, the results of Grumbach et al. suggest that, in each of these 14 cases, the locus for G-6-P.D. was active in only one X chromosome, and correspondingly inactive in all the remaining X chromosomes. These findings would appear to give further support to the theory that only one X chromosome is fully active in intermitotic nuclei.

Although the problems concerning the numerical correlation between sex chromatin bodies and X chromosomes have been fairly adequately solved by the above hypotheses, further points have arisen regarding the derivation and nature of the heteropyknotic X chromosome which forms the sex chromatin body.

It was postulated in 1959, by Ohno and his colleagues, that the heteropyknotic X chromosome in the female is paternally derived. However, as pointed out in the 'Lancet' (1960), there is the obvious difficulty that if this were the case, half the males would have one such X, and a quarter of the females would have two such X chromosomes.

Ohno and his colleagues later revised their opinion, and (Ohno and Hauschka, 1960), postulated instead that the heteropyknotic properties of the two X chromosomes within the same female nucleus alternate, depending on the stage of D.N.A. synthesis and the degree of overlapping in the replication of the two X chromosomes. Although this might appear to be a reasonable hypothesis, it does not, however, explain the uniform presence of small sex chromatin bodies in subjects with deletions of one of the X chromosomes as reported by Jacobs et al. (1961). In such cases, if the heteropyknotic properties of the two X chromosomes alternate, one would expect to find sex chromatin bodies of normal size derived from the normal X chromosome in addition to the small sex chromatin bodies derived from the deleted X chromosome.

A more satisfactory theory regarding the derivation of the heteropyknotic X chromosome has been proposed by Lyon (1961, 1962). From observations made in mouse genetic studies, Lyon deduced that in the female, at some time early in embryonic life, probably when sex chromatin first becomes visible, one X chromosome in each cell becomes inactivated and heteropyknotic. The inactivated X chromosome in each cell may be of either maternal or paternal origin, the choice being made at random by each cell

present in the embryo at that time. Once the X chromosome is inactivated, the inactivation remains fixed thereafter and is transmitted to all daughter cells.

Further evidence in support of this hypothesis that the inactivated X chromosome may be either maternally or paternally derived was independently provided by Boutler et al. (1962), who, working with the enzyme G-6-P.D. also arrived at the conclusion that "the human female is normally a genetic mosaic containing cells with a genetically active maternal X chromosome, and cells with a genetically active paternal X chromosome".

Although, in view of the above work, this part of Lyon's hypothesis may therefore be considered to be established, certain difficulties are encountered when the argument is applied to anomalies of sex chromatin and X chromosomes.

For example, if it is postulated that the 'Lyonised' X chromosomes are completely inactivated, then subjects with varying numbers of X chromosomes should show no abnormalities, and, individuals with the XXXXY, XXXY and XXY complexes would be the same as the normal XY male. The presence of various anomalies in the former three complexes, therefore suggests that part of each extra X chromosome must still be functioning, and

indeed, a recent elegant piece of work by Reed et al. (1963) on the sex-linked dominant blood group antigen Xg^a, described originally by Mann et al. (1962), has added strong evidence in favour of a segment of the 'Lyonised' X chromosome still remaining active.

It may therefore be concluded that in the male, the excess genetic material on any extra X chromosomes, in some way prevents the correct formation of the testis, and that the resulting abnormal gonad with the associated hormonal imbalance accounts for many of the abnormalities found in chromatin-positive Klinefelter's syndrome and its chromosomal variants. When the amount of excess genetic material is sufficiently great, as in the XXXXY male, additional somatic congenital anomalies are produced.

It is not known why some males and females with one extra X chromosome are mentally normal and others mentally sub-normal, but since mental defect may occur when either an extra X or an extra Y chromosome is present, as in the XXX, XXY and XYY complexes, it may be postulated that when mental deficiency does occur, the causal factor in each case is again due to the effect of excess genetically active material.

The preceding discussion indicates the way in which correlation of nuclear sexing studies and chromosome analysis in subjects with anomalies of the X chromosomes has led to many interesting deductions and hypotheses concerning the nature of the sex chromatin body. Nevertheless, although some of the problems concerning sex chromatin and X chromosomes have been resolved, many others remain. These include the different functions of the two X chromosomes in the human female, and the precise action of the X and Y chromosomes in sex determination.

Progress in human cytogenesis has only really gained momentum since 1956, when the techniques of chromosome analysis were brought to their present high standard. Painter (1923) forecast that "it is not to be doubted that in the course of time the individual chromosomes of man will come to be known quite exactly as we now know the chromosomes of insects". However, as Ford (1960) states, "The study of human cytogenetics has now attained the stage that the Drosopholists had reached about 40 years ago. We can hardly expect an advance as rapid and dramatic as they achieved in the twenties and thirties, particularly after the rediscovery of the salivary gland chromosomes. Nevertheless there is now a great interest on the

part of the medical profession and we can look forward to the screening of large numbers of individuals, at least in Western Europe and North America, and to a steady increase in our understanding of the relationships of chromosomal abnormalities to congenital disease, to neoplasia, and to general human biology".

SUMMARY

SUMMARY

The historical background of chromosome investigation and nuclear sexing is reviewed, and the normal human karyotype and sex chromatin findings in man are described. The principles of chromosome analysis and the techniques for the investigation of nuclear sex are indicated. An outline is given of the various fields of research in which nuclear sexing methods have been applied in man. There follows a discussion concerning the anomalies of nuclear sex and sex chromosomes which have been detected by these techniques in various intersex states.

The condition of chromatin positive Klinefelter's syndrome is then reviewed with particular reference to the incidence of the anomaly and its association with mental deficiency. The chromosomal variants of this syndrome are discussed in detail. Two cases, of the XXXY and XXXXY variants are described and compared with other similar chromosomal variants reported in the literature. These variants are reviewed and the effect of extra X and Y chromosomes are discussed with regard to the production of mental deficiency. Gonadal and somatic anomalies, and the formation of specific clinical syndromes are also considered. It is demonstrated that an increase in the number of

X chromosomes increases the severity of the gonadal abnormality, the degree of mental deficiency and the frequency of associated congenital abnormalities of the skeleton and soft tissues. It is shown that in the XXXXY chromosomal variant a clinical syndrome is produced distinct from the commonly encountered XXY form of the syndrome.

The results of two surveys of female mental defectives carried out by the buccal smear technique are given. In these, the incidence of the triple-X anomaly was found to be 0.67% in 595 inmates of a mental deficiency institution and 0.28% in 711 mentally handicapped schoolchildren. The results of surveys by other workers of the incidence of this chromosome anomaly in mental defectives and in the general population are then considered in relation to the above.

The clinical features of the six triple-X subjects detected in my two surveys are described, and it is shown that there are no features forming a specific clinical syndrome. Other cases of the triple-X anomaly which have been reported in the literature are reviewed, and the above finding confirmed.

The effect of extra X chromosomes in the female is discussed, and it is demonstrated that mental deficiency, although present in the majority of cases, is not a necessary

accompaniment of the triple-X anomaly. It is also shown that in some subjects, including one of my triple-X cases, fertility is not affected, and that the offspring need not be chromosomally abnormal. The effect of maternal age in the production of chromosomal anomalies is discussed.

Finally, the hypotheses concerning the derivation of sex chromatin and drumsticks are considered, and it is shown how the study of nuclear sex and sex chromosomes in normal and abnormal clinical conditions has led to the formulation of these present hypotheses.

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