STUDIES OF
HUMAN CHROMOSOMAL ANOMALIES
BY TISSUE CULTURE METHODS

A Thesis presented for

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by

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Studies of Human Chromosomal Anomalies by Tissue Culture Methods

SUMMARY

A description is given of the methods of culturing samples of bone marrow and peripheral blood material for the purpose of studying the human chromosome complement. The normal human karyotype is described.

An account is then given of the application of these methods to the investigation of the chromosome complement in patients with various conditions.

(a) Mongolism.

Studies were performed in twenty families where the mother was under 30 years of age when her mongol child was born. One translocation mongol was found; this translocation was thought to have arisen spontaneously, since both parents had normal karyotypes. One mother was found to be a mosaic of a normal cell-line and a cell-line trisomic for chromosome 21. Studies were also carried out on two families with two mongol children, but no translocation was found; on two mongols with a higher I.Q than average, both of whom proved to be trisomic for chromosome 21 and on five children where the diagnosis of mongolism was in doubt. Three of these had normal karyotypes, while the other two were trisomic for chromosome 21.

(b) Abnormalities of the sex chromosomes.

Chromosome studies were performed on four adult patients in a hospital for the mentally deficient who had been found to have two sex chromatin bodies in a proportion of cells in smears from their buccal mucosa. All had 47/XXX chromosome constitution.

Among 12 patients studied who had chromatin positive Klinefelter's syndrome, three were found to have an unusual chromosome constitution. They had karyotypes XX, XXY/XXXY, and XXXXY respectively. The remaining patients had the typical 47/XXY chromosome constitution. Three patients with chromatin negative Klinefelter's syndrome had a 46/XY chromosome complement

Studies were carried out on nine cases or doubtful cases of Turner's syndrome. Two of these patients were mosaics of two cell-lines; the first was an XO/XX mosaic, the second had an XO cell-line and a cell-line containing a normal X chromosome and an isochromosome for the long arm of the X. Two other individua had an XO karyotype, while the remainder had a normal female complement.

(c) Various pathological conditions.

Chromosome studies were carried out in 43 patients with a variety of conditions where it was thought that an abnormality might be present. These included genetically inherited conditio

other distinct syndromes, multiple congenital abnormalities and diseases of the blood. One case with multiple congenital abnormalities had trisomy of chromosome 18, and one case of chronic myeloid leukaemia had an XO cell-line in the bone marrow, with no Philadelphia chromosome present.

(d) Patients receiving ^{131}I therapy.

Chromosome studies on 19 patients receiving ^{131}I therapy suggested that this treatment is accompanied by some increase in the number of abnormal cells in the peripheral blood. There seemed to be no evidence that any particular chromosome or chromosome region was preferentially involved.

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CONTENTS

Papers included

The XXX Syndrome. Frequency among Mental Defectives, and Fertility.

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INTRODUCTION

Historical Background

The question of heredity has occupied men's minds since ancient times. It is mentioned in the works of many writers, among them Lucretius and Aristotle, but until the middle of the nineteenth century it remained a subject shrouded in superstition and speculation. Since then patient research has slowly uncovered the facts, although many of them were at first accepted with reluctance. The present state of knowledge of the chromosomes as the material basis of inheritance is the result of the coordination of various lines of study, most of which were made possible by the invention of the microscope.

By the time fertilisation was observed in the sea-urchin by Hertwig (1875), the cell theory as formulated by Schleiden and Schwann (in Swanson, 1960) was already established, and it had been pointed out (Virchow, 1858) that cells could arise only by division of pre-existing cells. Thus the idea of heredity as a consequence of genetic continuity of cells by division was introduced. Hertwig (1875) showed that fertilisation involved union of the nucleus of the sperm with that of the egg, and two years later Strasburger

(1877) observed the same phenomenon in plants. Attention was thus focussed on the nucleus.

The bodies which are now known as chromosomes were first recorded by Naegeli (1842) and Hofmeister (1848) in studies of nuclear division in pollen mother cells in Tradescantia. After oil immersion techniques were introduced by Abbé in 1873, much more detailed studies became possible (in Sirks, 1952). The name chromosome (from the Greek chromos = colour, soma = body) was first introduced because of the marked ability of these bodies to take up stain (Waldeyer, 1888).

Flemming (1882) described the details of the mitotic figure; he also discovered that the chromosomes split in a longitudinal fashion during cell division. Van Beneden (1884) and Heuser (1884) showed that the longitudinal halves of the chromosomes passed to the daughter nuclei during division. The first hypothesis that these chromosomes might constitute a linear arrangement of basic units representing hereditary properties was put forward by Roux in 1883.

About the same time van Beneden (in Wilson, 1925) showed that in fertilisation the chromosomes of the offspring are derived in equal number from the nucleus of egg and of sperm.

Weismann was the first to suggest that the production of germ cells involved a reduction or halving of the chromosome number in the nucleus. It was thought at first that this might involve degeneration, or casting out of half the chromosomes, but von Winiwarter (1901, 1909) was able to show otherwise: what had appeared to be a longitudinal split in a single chromosome was in fact the separation of two chromosomes which had been associated side by side, and was thus a mechanism of reduction.

Earlier workers, while appreciating that the number of chromosomes per nucleus was constant for any given species, had assumed that all of these chromosomes were alike in both morphology and physiology. That chromosomes are of different shapes and sizes, and that these differences are constant in any species, was recognised by Montgomery (in Wilson, 1925). He postulated also that the separating pairs observed by von Winiwarter were each composed of one chromosome that was of paternal origin and one that was of maternal origin, and that the two were homologous. Qualitative differences in chromosomes were demonstrated by Boveri (1902, 1907) in his fertilisation experiments in sea-urchins.

1900 marked the rediscovery of the work of Mendel describing the segregation of genetic determinants influencing inherited characteristics in sexually-reproducing organisms. Mendel showed, in experiments in which he crossed two strains of garden peas breeding true with respect to a given characteristic (e.g. tall x short plants), that such characteristics are determined in an individual by two factors, which may or may not be the same, and that at the production of germ cells gametes are produced which have either one factor or the other. He showed also that where two different characteristics are concerned the factors segregate independently of one another. These factors were later given the name gene by Johansen (1909).

Sutton (1903) was the first to point out the correspondence between the behaviour of the chromosomes in mitosis (cell division) and meiosis (reduction division) and the behaviour of the Mendelian genes in inheritance. The chromosome theory of inheritance has developed from this idea. Significant advances have come from the work of Morgan and his group of co-workers in their breeding experiments on Drosophila (Morgan et al., 1915). They found, contrary to Mendel's second law, that certain

characteristics tended to be inherited together. All the many characteristics which can be studied in Drosophila fell into four such groups, which they called linkage groups, and these were interpreted as corresponding to the four chromosome pairs. Further, they were able to construct maps showing the order in which the genes were linearly arranged along each chromosome. For each gene situated along the length of each chromosome of any pair there is a corresponding gene at the same position on the homologous chromosome of that pair influencing the same characteristic or characteristics, though not necessarily in the same way.

Sex Chromosomes

For centuries it was believed that external conditions could influence the sex of an offspring (in Darlington, 1952). Mendel suggested that sex might be a hereditary phenomenon, a hypothesis which was confirmed by the work of Correns on fertilisation experiments with Bryony plants (1907).

It is now realised that sex determination is a complex mechanism with a chromosomal basis. In the most general situation there is one pair of chromosomes in which there is a homologous region, but also a region in which each chromo-

some carries genes not represented on the other. In one sex, one of these chromosomes is represented twice (usually referred to as the X chromosome), and the other member of the pair is not present at all. At meiosis only one type of gamete can be produced (X-bearing), and this is therefore called the homogametic sex. In the other sex each of the chromosomes is represented once (one X and one Y). Two types of gamete can be produced at meiosis, hence this is known as the heterogametic sex. In most groups of plants and animals the male is the heterogametic sex, although in some groups it is the female.

The differential region of this pair of chromosomes is sometimes more developed in one member than in the other, so that there is a visible cytological difference between X and Y; the homologous region may be very much reduced. There are some groups in which the Y chromosome is missing altogether, so that the cells of the heterogametic sex have one chromosome fewer than those of the homogametic one. This is referred to as an XX:XO type of sex determining mechanism.

It is probable that not all sex determining genes are carried on the sex chromosomes; similarly not all the genes

on these chromosomes are concerned with sex development. Genes carried on the non-homologous region of either X or Y show a very particular type of inheritance pattern known as sex linkage.

Human Chromosomes

The difficulty of obtaining suitable material for the study of chromosomes in man (see section on Technique) was probably responsible for the lack of interest in the subject in the early part of this century. Von Winiwarter (1912), working on sectioned testicular material, declared that the chromosome number in the human male was 47, and that there was an XX:XO sex determining mechanism. Painter (1923) claimed that there was a Y chromosome and that the number in both sexes was 48. Until 1956 this was the accepted human chromosome number and the only point of discussion was the nature of the sex chromosome constitution. The improvement in techniques at this time led to the discovery by Tjio and Levan (1956) that the true number was in fact 46 with XY sex chromosomes. The present period of intense investigation of the chromosome complement in normal and abnormal human individuals was thus initiated. Since then the only author

to disagree has been Kodani (1957, 1958), who has reported the three chromosome numbers 46, 47 and 48 to be present in testicular material among Japanese males. Since this has not been confirmed by other workers on Japanese individuals (Makino and Sasaki, 1961; Makino et al., 1962; Makino, Yamada and Sofuni, 1963), there is some doubt about the accuracy of the observation, although it has been suggested that there may be supernumerary chromosomes present (Stern, 1959).

Chromosome Chemistry

Chromosomes are known to be composed of two nucleic acids (desoxyribose nucleic acid [DNA] and ribose nucleic acid [RNA]), two main types of protein and a small amount of calcium (White, 1961), but how these combine to form the visible chromosome is not yet certain. The evidence at present points convincingly to DNA as the main carrier of genetic information (Ephrussi-Taylor, 1951).

DNA is a long, fibre-like molecule, an unbranched chain composed of many nucleotide units. Each of these consists of a molecule of phosphoric acid, a pentose sugar (d-2-desoxy-ribose), and a nitrogenous base; the consecutive nucleotides

are joined by bonds between the sugar and the phosphate molecules. There are four bases which are commonly found, adenine and guanine (purines) and thymine and cytosine (pyrimidines), and they can occur in any sequence along the chain. The number of adenine bases is always equal to the number of thymine bases, similarly the number of guanine always equals the number of cytosine; the ratio adenine + thymine to guanine + cytosine is constant within a species but differs widely between species.

The interpretation of the structure of the DNA molecule which is now accepted was proposed by Watson and Crick (1953). They state that the DNA molecule is composed of two parallel strands which are coiled round a common axis to form a double helix; the sugar-phosphate backbone is to the outside and the nitrogenous bases to the inside. The bases lie perpendicular to the axis of the fibre and are held together in pairs, one from each chain, by hydrogen bonds. This pairing is very specific; it can occur only between adenine and thymine, and between cytosine and guanine. Thus the two chains comprising the molecule are complementary to each other.

This structure provides for one of the two essential requirements of a carrier of genetic information, namely the

exact self-duplication of the molecule. Watson and Crick suggest that each chain of the molecule serves as a template upon which a new complementary chain is built up. This reduplication takes place in the interphase nucleus before the beginning of cell-division; the phenomena observed in cell-division are the processes of mechanical separation of the doubled chromosome.

The Coding Problem

The second requirement of a genetic material is that it should be able to exert a very specific influence on the cell, and there is now evidence that this is done by influencing the synthesis of specific proteins (Symonds, 1962). It is thought that DNA exerts this influence on proteins by controlling the sequence of their component amino-acids along the polypeptide chain. This control must be influenced by the sequence of the bases along the DNA molecule, since this is the only source of variation within the molecule. Since there are only four bases, and twenty amino-acids are commonly found in proteins, it is argued that the sequence of the four bases must in some way form a code for the sequence of the amino-acids. The present conception of this code is due to the work of Crick *et al.* (1961), who suggest that a group of

three (or a multiple of three) bases codes one amino-acid, and that there is no overlapping between the groups. Since there are sixty-four possible triplets, and only twenty amino-acids, it is likely that more than one triplet can code for one amino-acid. The sequence of bases must be read in order from a fixed starting point; if this point is displaced the grouping of the bases into triplets is disarranged and the reading becomes incorrect. Similarly if at any point along the code a base (or bases) is added or deleted, the reading of the code from that point onwards will be incorrect.

Some Other Aspects of Chromosome Studies

A great deal of work is being done at present on many different aspects of chromosomes, and these can be mentioned only briefly here. The classical approach continues to reveal the chromosome constitution of species not yet investigated in this way (Walen and Brown, 1962; Lu and Brodie, 1962; Shaw and Krooth, 1964). The improvements in technique which facilitated the human studies have also allowed study of other species which present much the same technical difficulties, e.g. gorilla (Hamerton et al., 1961; Chiarelli, 1961).

Many approaches to the fine structure of the chromosome are being pursued. Use has been made of naturally occurring giant chromosomes - the lampbrush chromosomes in the oocyte of the newt have been investigated (Callan and Lloyd, 1960), and DNA synthesis has been studied in the polytene chromosomes of salivary glands in the Diptera (Rudkin and Corlette, 1957). Electron microscope techniques are being employed (Yasuzumi and Sugihara, 1961), and biochemical methods are being used to investigate the problem of coiling and of the arrangement of the various molecules within the chromosome (Cole, 1961; Gall, 1963). Organisation and duplication of chromosomes is being studied by autoradiographic techniques (Taylor, Woods and Hughes, 1957) which are also proving of use in identifying individual chromosomes (Rowley et al., 1963; Yunis, Hook and Mayer, 1964). Other functions of DNA in the cell are being investigated biochemically (Allfrey and Mirsky, 1957).

The production of mutations and of gross chromosomal aberrations by artificial means is giving important results. Ionising radiation has been studied in this respect (in Lea, 1955), also chemical agents such as DNA inhibitors (Kihlman, Nichols and Levan, 1963) and alkylating agents (Koller,

1957-58), and infection with virus particles (Wolman, Hirschhorn and Todaro, 1964). Research into the relationship of chromosomes to neoplastic disease is of the greatest importance at present, and much work is being done in the fields of both tumours and the leukaemias (Spriggs, Boddington and Clarke, 1962; Nichols, 1963).

The study of mammalian species can present many problems: for example, there are often large numbers of small chromosomes. Man in particular, with the long space of time between generations, the small number of offspring and the absence of experimental breeding, is especially difficult to investigate. Hence the study of human chromosomes is still in its infancy, and the majority of experimental work is done on more favourable species.

However, the problem of mapping the human chromosomes has recently begun to be elucidated. Sex-linked genes such as those responsible for haemophilia and colour-blindness have been familiar for some time, and recent evidence has shown that the Xg blood group genes are located on the short arm of the X chromosome (Lindsten et al., 1963). Studies have now shown that none of the X-borne genes responsible for haemophilia (VIII) (O'Brien et al., 1962), Duchenne muscular

dystrophy (Clark et al., 1962), and the protan and deutan types of colour-blindness (Jackson, Symon and Mann, 1962) are close to the gene locus of the Xg^a blood group. Other data show close linkage between gene loci for glucose-6-phosphate dehydrogenase and colour-blindness (Jackson, Symon and Mann, 1962), and loose linkage of glucose-6-phosphate dehydrogenase and Xg^a loci (Adam et al., 1962). Some linkage groups not on the sex chromosomes are also beginning to be recognised (Renwick, 1961).

Objects of the Present Study

The first object was to establish in the laboratory the recently discovered techniques involved in the study of human chromosomes from samples of bone marrow and peripheral blood, techniques by which relatively large numbers of individuals could conveniently be screened. These methods have since been adopted widely and successfully; however, when the present work was begun at the end of 1959, they were in their infancy. In fact there was only one other laboratory of this kind in Scotland, that of Court Brown and Jacobs in Edinburgh.

Until the middle of 1962 the author was the only worker in Glasgow studying human chromosomes by these methods. The

result was that, as chromosome studies became clinically more important, much time had to be spent in the investigation of cases as they presented themselves in hospital. These clinical demands made it difficult to pursue a consistent programme of research, but on the other hand they presented opportunities for making new observations and confirming others which were still in doubt at the time.

The chromosomes of the patients described in this thesis were examined by the author. About 4 out of every 5 cultures initiated were successful, and a total of 12,553 cells from 273 patients has been studied.

The individuals studied fall into the following categories:

(1) Apparently normal individuals, studied for the purpose of becoming more familiar with the normal human karyotype, and of obtaining information about the range of variation, both in size and in percentage of aneuploid cells, within the normal limits.

(2) Individuals with various pathological conditions were investigated to see whether any visible abnormality of the chromosomes was present.

(3) Patients with abnormalities of the sex chromosomes were studied with the purpose of supplementing, and if possible extending, the present state of knowledge.

(4) In a few cases cytogenetics has been used as a tool to help confirm a doubtful clinical diagnosis in conditions where a chromosome abnormality is well established, e.g. mongolism. Similarly, parents of mongol children have been studied, to try to discover any with chromosome constitution which would give them a higher risk of producing another such child.

(5) Patients treated with ^{131}I were examined for the presence of radiation induced chromosomal aberrations in the cells of their peripheral blood.

SECTION I

TECHNIQUE

Introduction

The first essential in studying chromosomes is to have a sufficient number of intact cells for analysis. They should be well spread out so that the chromosomes lie in one plane and overlap as little as possible. The chromosomes should be well fixed so that they are sharply defined and not fuzzy in outline. A suitable cell is shown in Fig. 1.

The practical difficulties of obtaining suitable preparations were the main cause of the early confusion among workers on human chromosomes, who had to use tissues naturally dividing in the body. The initial observations were made on testicular material from executed criminals, and as this had not always been removed from the body immediately after death, post-mortem changes giving rise to clumping added to the difficulties. The observations of Painter (1923) and von Winiwarter (1912) were made on fresh testicular material but still employed the method of embedding the specimen in paraffin wax after fixation, and then sectioning. When using thin sections it is difficult to be certain that all the chromosomes

Figure 1. Mitotic figure from a normal male. Shows also the presence of satellites (arrowed), and the association often seen between two or more of the satellited chromosomes.

of any one cell are seen in any one section, while increasing the thickness of the sections decreases the possibility of seeing the chromosomes all lying in the one plane, and also causes optical difficulties.

The method of handling testicular material described by Ford and Hamerton (1956), involving the use of hypotonic solution before fixation, followed by manual squashing of the tubules, results in much better dispersion of the chromosomes. However the occasions on which testicular material can be obtained are limited, since specimens can only be obtained when this is justified on clinical grounds.

The development of long-term tissue culture techniques, providing cells which can either be examined as a monolayer, or be easily brought into suspension, was of great advantage to cytologists. Cultures can be obtained from a wide range of tissues, both embryonic and adult, including skin, fascia lata, lung, heart, liver and spleen. Using these methods Tjio and Levan (1956) established the human chromosome number as 46. These authors incorporated both the use of hypotonic treatment (accidentally discovered by Hsu, 1952) to swell the cells and help the spreading of the chromosomes, and the use of colchicine - first used by botanists to produce polyploid

cells (Swanson, 1963) - to inhibit spindle formation, and thus help to accumulate mitoses. These methods yield excellent results and are at present widely used. There may, however, be a delay of several weeks for the cultures to grow sufficiently before cytological examination is possible. Since each cell divides frequently during this growth period, there is also the possibility of an abnormal cell-line arising *in vitro*.

The short term tissue culture methods to be described below have facilitated the present-day large scale examination of human chromosomes. Of such methods, the bone marrow method of Ford, Jacobs and Lajtha (1958) was used initially in the present study. Overnight incubation of this rapidly dividing tissue is sufficient, and the mitoses observed are the first division of the cell *in vitro*. The peripheral blood method described by Hungerford *et al.* (1959) was later adopted because of the ease with which specimens can be obtained from the patient. Mitosis is initiated by the use of the substance phytohaemagglutinin, and again there is evidence that it is the first *in vitro* division which is observed after the three day incubation period (Bender and Prescott, 1962).

The first object of the work described in this thesis was to establish these short-term methods as a routine procedure. When the first cultures were prepared in this laboratory, during December 1959, the bone marrow method was the only one available; moderate success was achieved. However, almost immediately Hungerford's recently-published method of culturing leukocytes began to supplant it. The first culture using this method was prepared towards the end of January 1960. For about two months the two were run in conjunction, but from April 1960 the leukocyte culture method only was used, because it proved more reliable and gave better preparations. It is much less unpleasant for the patient concerned, and hence much more suitable for investigating relatively large numbers of individuals.

Culture of Bone Marrow Cells for Chromosome Investigation

[Ford, Jacobs and Lajtha, 1958] (Solutions used - see Appendix A)

About 2 ml. marrow is withdrawn from the patient, usually from the sternum. This is injected into 18 ml. sterile Ringers solution to which anticoagulant has been added to prevent clotting (100 i.u. Heparin/ml.). The specimen is

placed in a centrifuge within half an hour, and is spun at 1400 r.p.m. for 15 minutes; all the supernatant liquid is then discarded and replaced by 0·5 ml. glucose-saline solution (0·6 gm. D-glucose and 0·7 gm. NaCl per 100 ml. doubly distilled water). The suspension is well mixed, and, after a nucleated cell count has been performed, is diluted to the optimum white cell concentration of about 10,000/mm^3. The diluent is a mixture of 1 part by volume of glucose saline to 3 parts AB positive serum, or the patient's own plasma. It is then divided into 2 ml. aliquots in sterile bottles and incubated overnight at 37°C. Colcemid, at a final concentration of 4×10^{-6} gm. per 1 ml. of suspension is added before incubation.

The cell suspension is then transferred to a centrifuge tube and spun at 800 r.p.m. until all the cells have settled. The supernatant liquid is discarded and replaced by 2 ml. of 0·95% sodium citrate in which the cells are incubated at 37°C for 15-20 minutes. They are then spun as before, the supernatant liquid is removed, and the cells are fixed by the addition of approximately 2 ml. of a mixture of 3 parts by volume of absolute alcohol to 1 part of glacial acetic acid. The fixative must be added slowly with constant agitation of

the cells, so that an even suspension without clumping of the cells is obtained. The cells are left in this fixative for 30-45 minutes at 0°C. They are then suspended successively in 75% (by volume) alcohol, 50%, 30% alcohol and distilled water. From this they are transferred to N HCl and hydrolysed at 60°C for 4 minutes; the hydrolysis is stopped by cooling the tubes quickly. The acid is discarded and the cells are suspended in Feulgen's reagent, where they are left for 1 hour in the dark. Finally they are washed in a sulphurous acid rinse and suspended in 45% by volume acetic acid.

A small drop of this suspension is placed on a clean slide, covered with a siliconised coverslip, and pressed by hand. Considerable pressure is sometimes required to achieve spreading of the chromosomes, and it is essential, in the first place, that the coverslip should be perfectly flat, and, in the second place, that it should not move sideways during this stage. The slide is then placed on solid CO_2 and the coverslip removed; the cells are additionally stained by flooding the slide with 1% orcein in 45% acetic acid for 5 minutes. The slide is then passed through two changes of cellosolve and one of euparal essence before mounting in euparal.

Culture of Leukocytes from Peripheral Blood (for Chromosome Investigation) [Hungerford et al., 1959]

Between 10-20 ml. peripheral blood are withdrawn from the patient and placed in a sterile 1 oz. universal container bottle containing 2 ml. 0·2% Heparin solution. 0·4 ml. phytohaemagglutinin is added, the bottle shaken to mix the contents well and then left to stand for 30-45 minutes at $0^{o}C$. The phytohaemagglutinin has a dual function; in the first place it agglutinates the red cells and thus facilitates the separation of leukocytes for culture, and in the second place it stimulates mitotic activity in the separated leukocytes.

After being centrifuged at 350 r.p.m. for 3-4 minutes, the supernatant plasma containing the leukocytes is removed. A nucleated cell count is performed, and the plasma is diluted with a commercial tissue culture fluid (T.C. 199) to a final concentration of 1000-1200 cells per mm^3. It has been found that good growth is not obtained if the concentration of cells is over 1400-1500 per mm^3. The culture is then divided into 10 ml. aliquots in sterile 1 oz. bottles, and incubated at $37^{o}C$ for 72 hours.

Two hours before the end of the incubation period colcemid is added to each culture to give a final concentration

of 4 x 10^{-6} gm./ml. This treatment accumulates sufficient mitoses for examination and yet avoids the contraction of chromosomes which prolonged exposure to colcemid produces.

After incubation has been completed, the suspension is transferred to a centrifuge tube and spun at 350 r.p.m. for 5 minutes; the supernatant liquid is discarded and replaced by a hypotonic solution. The author found 4 ml. of Hanks balanced salt solution, diluted 1 part by volume of Hanks to 3 parts of distilled water, to be the most satisfactory; the cells are left in this solution for 6 minutes at 37°C. This is sufficient to achieve spreading of the chromosomes without loss of definition, and without breaking the cells. The cells are then fixed in 2 ml. of a mixture of 1 part by volume of glacial acetic acid to 3 parts absolute alcohol, and left overnight in this fixative at 0°C.

Immediately before the slides are made the cells are re-suspended in 1-2 ml. of fresh fixative. One drop of this suspension is placed on a clean wet slide and dried quickly over a spirit lamp. The slide is then stained for 45 minutes in orcein (2 gm. natural orcein in 100 ml. of 60% acetic acid - this and the following percentages are by volume). After a quick rinse in 50% acetic acid to remove excess stain,

the slides are taken through two changes of 95% alcohol in which they remain for two minutes each, transferred to absolute alcohol for 15 seconds, and then left in xylol for about 15 minutes. Finally the cells are mounted in a suitable mounting medium, the one in current use being Harleco. This medium was found more satisfactory (than DePeX) when examining the cells under the phase contrast microscope, which was occasionally used when the chromosomes were too lightly stained. Initially, Feulgen's reagent was used in addition to the orcein staining, but it was found that very satisfactory results were obtained without it.

Notes on the Author's Modifications of Leukocyte Technique

1) Concentration of cells and percentage of plasma

According to Hungerford's description for preparing these cultures, the leukocyte concentration is determined and the plasma containing them in suspension is recentrifuged. The cells are then re-suspended in a mixture of 10-20% normal human plasma in T.C. 199, the final concentration being 1-2 x 10^6/ml., i.e. 1000-2000/mm^3.

In this laboratory the patient's own plasma was used in place of normal human plasma. The leukocyte suspension was

diluted with T.C. 199 until the desired concentration was achieved. Varying the concentration of cells in a series of cultures derived from the one individual showed that better growth was obtained at a concentration of 1000-1200/mm^3 than at 1800-2000/mm^3. So long as approximately this concentration of cells was achieved, experience showed that the proportion of plasma could vary from 10-40% and still give good results.

2) Phytohaemagglutinin

During 1960 no trouble was encountered with the phytohaemagglutinin, but early in 1961 a new batch was received with apparently greatly increased agglutinating properties. As a result, the white cells were carried down with the red cells and it was impossible to obtain sufficient leukocytes to prepare a culture.

A series of dilutions of this phytohaemagglutinin in Hanks B.S.S. was prepared ($\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$). Six 5 ml. samples of blood were obtained, and to each was added 0·1 ml. of one of these dilutions. The two dilutions $\frac{1}{8}$ and $\frac{1}{16}$ were found to give the best yields of both plasma and leukocytes. Consequently the original phytohaemagglutinin was diluted

10-fold; cultures prepared using this strength showed a satisfactory proportion of cells in mitosis. All subsequent batches of phytohaemagglutinin were tested for strength, and the 10-fold dilution continued until 1963, when the Wellcome product became available. A trial run used according to the makers' instructions gave excellent results.

3) Colchicine treatment

Hungerford pointed out in 1959 that optimal mitotic activity occurred after 3-4 days incubation at 37°C, and recommended the addition of colchicine (final concentration 1×10^{-7} M) 17-19 hours before harvesting to accumulate large numbers of cells in mitosis. However, since prolonged exposure to colchicine causes over-contraction in mitotic chromosomes, a shorter period of colchicine treatment seemed advisable. On several occasions a series of identical cultures was set up from an individual, and exposed to colchicine (final concentration 4×10^{-6} gm./ml.) in the following manner:

(i) 17 hours incubation at 37°C with colchicine, following incubation for 49 or 51 hours without.

(ii) 2 hours incubation at 37°C with colchicine, following incubation at 37°C without it for 35, 65, 66, 70, 71, 72, 89, 93 or 95 hours.

Of these variations, 65-66 and 71-72 hours incubation, followed by 2 hours colchicine treatment, gave the most satisfactory results.

Parallel cultures from one individual were also treated with colchicine at a final concentration of 4×10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} gm./ml., in an attempt to find the most suitable strength to accumulate mitoses without contracting the chromosomes. A concentration of 4×10^{-6} proved the most effective.

4) Method of preparing slides

Initially when using the leukocyte culture method, the cultures were harvested on completion of the colchicine treatment. The same method was used as for the bone marrow cultures, i.e. after hypotonic treatment and fixation, the cells were stained in Feulgen's reagent and slides were prepared by the squash technique. This method is not entirely satisfactory as considerable pressure may be required to spread the cells.

Cultures being harvested were divided into two equal portions; the first was treated by the usual method while the second, after fixation, was treated by a modification of the air-drying method of Rothfels and Siminovitch (1958). 1-2 drops were placed on a clean wet slide and dried gently over a low flame. The preparations thus obtained were of a higher quality than those prepared by the squash technique.

The advantages of the air-drying method of preparing slides as compared with the squash technique are two-fold. In the first place it achieves better spreading of the chromosomes. There is less overlapping and the morphology of the chromosomes is more readily distinguished in the majority of preparations. In the second place it avoids damaging the cells to the same extent; thus the number of aneuploid cells caused by handling of the material is decreased, and the counts obtained may be regarded as giving a truer reflection of the chromosome constitution.

5) Hypotonic treatment

In order to improve the spreading of chromosomes using the air-drying technique, the use of diluted Hanks B.S.S. for the hypotonic treatment as recommended by Moorhead et al. (1960)

was investigated. Again cultures being harvested were divided into two equal portions; the first was treated with citrate as before, the second with Hanks B.S.S., diluted 1 in 4 or 1 in 10 with distilled water, for periods between 5-20 minutes. 6 minutes exposure to 1 in 4 dilution of Hanks B.S.S. achieved improved spreading.

Most of the modifications 1) to 5) were subsequently described by Hungerford and his colleagues in the improved version of their method published later in 1960 (Moorhead et al., 1960).

Examination of Preparations

The preparations thus obtained were examined under the microscope, a process which, in a patient with straightforward chromosomes, occupies 1-2 days. The number of chromosomes present was counted in sufficient mitoses to establish the modal number in the marrow or blood cells of the patient, and to eliminate the possibility of mosaicism. Originally the aim was to count between 20-30 cells per patient, and this was later extended to 50, which is now the routine target where no second cell-line is suspected. In addition a number of cells (originally 3-4, now 6-8) were fully analysed

under the microscope to determine the chromosome constitution, i.e. the distribution of the chromosomes among the various sub-groups was ascertained (see Section II). Many investigators tend to rely on photographs, but it was found in the course of the present study that, with practice, the simple, economical and rapid method of direct microscopy was sufficient in most cases, although where there was any uncertainty the cells were photographed and karyotyped. In addition, all non-modal cells were analysed microscopically so that any evidence of mosaicism would be detected. The distribution of all modal and non-modal cells is shown in Table I, excluding those from mosaics and from patients who had been irradiated. To illustrate the improvement in technique, the bone marrow results are compared with the results from 100 recent blood cultures.

Cultures were prepared from 341 individuals, not including those receiving ^{131}I therapy; 46 of these were on bone marrow material, and the remaining 295 were on peripheral blood. Of these, 15 of the bone marrow cultures (approximately 33%) and 236 of the blood cultures (80%) were successful, and from these 251 individuals a total of 9553 cells was studied.

TABLE I

Distribution of Modal Cells

Material	% Cells with Chromosome No. M-1	M	M+1	Total Cells Counted
16 patients - bone marrow material	10	84	6	223
100 recent patients - peripheral blood material	5	94.5	0.5	4348
All patients, present study - excluding mosaics	6	93	1	8022

M = modal number

SECTION II

IDENTIFICATION OF NORMAL HUMAN CHROMOSOMES

After the development of the new techniques previously described, the human chromosome number was established (by Tjio and Levan) as 46; 22 pairs of autosomes and one pair of sex chromosomes. A system of nomenclature had then to be established. The longest chromosome is 7-8 μ in length and the shortest c. 1·5 μ (Ferguson-Smith, 1962). In the rather artificial mitotic metaphase produced by these techniques, each chromosome is seen to be longitudinally split into two chromatids joined at one point, the centromere. This may be situated in the middle of the chromosome, dividing each chromatid into two more or less equal arms (metacentric), or may be nearer one end than the other, forming a long and a short arm on each chromatid (submetacentric). In some cases it is found very near one end, so that the short arm is very short indeed (acrocentric).

The term karyotype is applied to the chromosomes of a single mitosis arranged in order of decreasing length and relative centromere position, and this term has the extension in meaning that the chromosomes of a single cell can typify

those of an individual (Denver Report, 1960). The term idiogram refers to a diagrammatic representation of the karyotype. Among the first authors to publish idiograms of the human complement were Ford, Jacobs and Lajtha (1958), Tjio and Puck (1958), Levan and Hsu (1959) and Chu and Giles (1959). These idiograms were constructed from measurements of lengths and arm ratios made either from photographs or from camera lucida drawings: one of the main points of difference among them was in the designation of the various groups.

In 1960 a Human Chromosomes Study Group met at Denver to eliminate this confusion in nomenclature and evolve a standard system which would be acceptable to the majority of workers. It was agreed that the autosomes should be numbered 1-22, as nearly as possible in order of decreasing length, while the sex chromosomes continued to be referred to as X and Y. The 22 autosomal pairs were divided into 7 groups which can readily be distinguished from one another. It was recognised that considerable difficulty is experienced in attempting to identify the individual chromosome pairs within some of the groups; the present arrangement is still somewhat tentative and varies from one laboratory to another,

while Patau claimed at one time that it was impossible to distinguish further than the 7 groups with the information available.

Recently Ferguson-Smith *et al.* (1962) have described the occurrence of secondary constrictions as an aid to identification. These are regions of heterochromatin which have staining properties different from the rest of the chromosome (see Fig. 2). They are most frequently found on the short arms of the acrocentric chromosomes, where they produce the characteristic satellites. Since satellites have been observed on all the acrocentric autosomes they are of little value in identifying the various pairs, but secondary constrictions are also observed with varying frequency at other sites. Those on chromosome pairs 1 or 16, for example, are again of little value in identification, since both pairs are recognisable without them. It is important, though, to recognise the secondary constriction as such, since its presence may alter the apparent length and arm ratio, and lead to misidentification. Within the 6-12 group (see below) secondary constrictions may be of value in recognising individual pairs. The idiogram of Ferguson-Smith *et al.* (1962), incorporating these constrictions, is the one

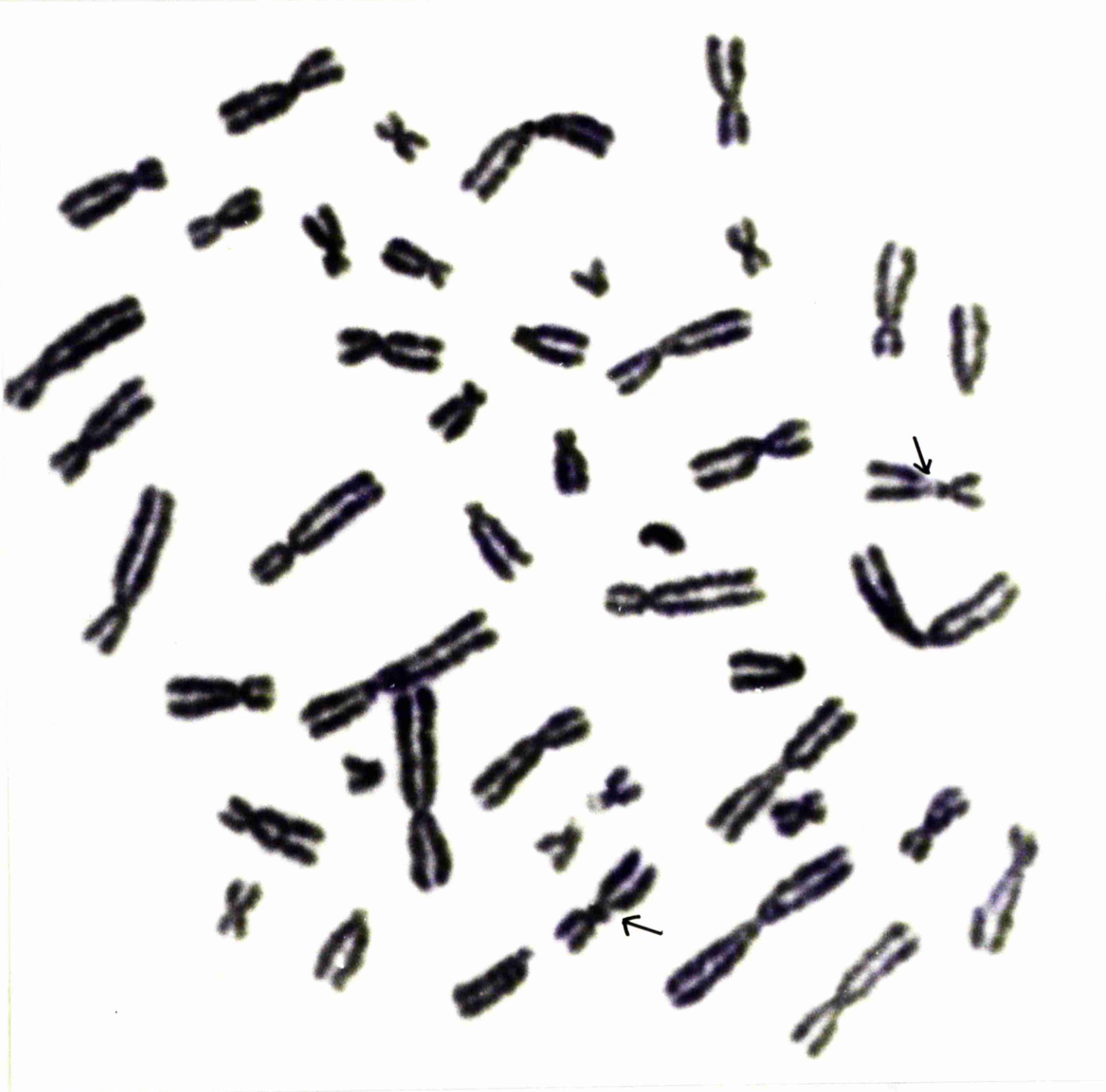


Figure 2. Mitotic figure from a normal male, showing secondary constrictions (indicated by arrow).

adopted for use in the present study (see Fig. 3). A recent Ciba Foundation Guest Symposium on the Normal Human Karyotype (held in London in 1963) is in substantial agreement with this arrangement, with the exception that the position of pairs 8 and 9 is reversed.

Summary of Groups

Chromosome pairs 1-3 (A). These three largest pairs are readily recognisable: 1 and 3 are metacentric, 2 submetacentric.

Pairs 4 and 5 (B). These two pairs are submetacentric, with fairly small short arms, and are very similar to each other.

Pairs 6-12 and X (C). In this group of medium sized chromosomes (15 in the male, 16 in the female) it is difficult to identify the pairs exactly. Four pairs of the autosomes in this group are more metacentric than the other three pairs, and these are designated by Ferguson-Smith et al. (1962) as 6, 7, 9 and 11. The X chromosome also lies in this subgroup. It is generally accepted that it lies between pairs 6 and 7 in size, and is rather more metacentric than either. Chromosome number 9 in this classification can frequently show

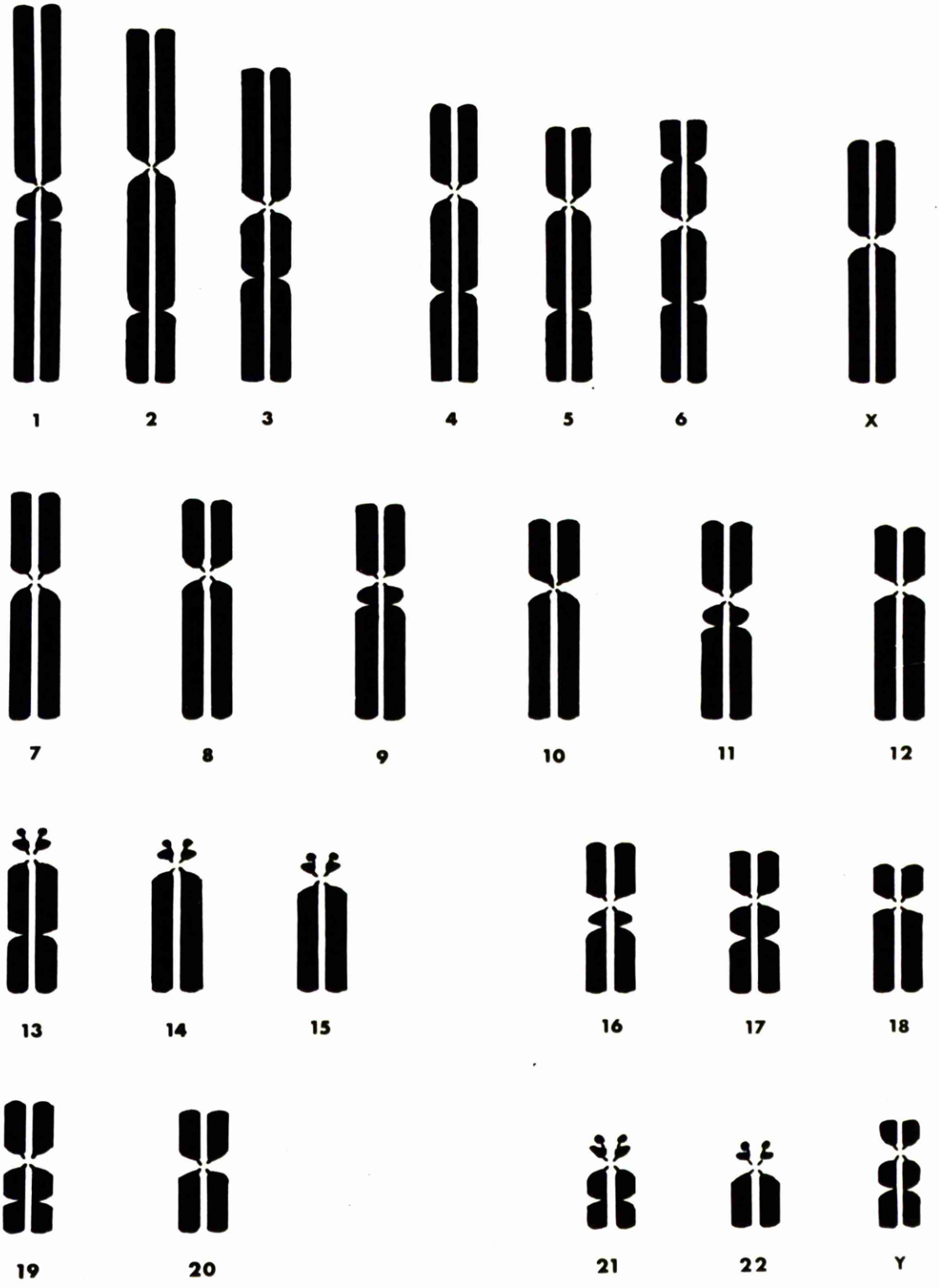


Figure 3. The normal human idiogram. Reproduced by kind permission of Dr. M.A. Ferguson-Smith.

a secondary constriction on the long arm near the centromere. The three more submetacentric pairs are designated 8, 10 and 12.

Pairs 13-15 (D). This group consists of the six large acrocentric chromosomes. These are all very alike, and sometimes satellited.

Pairs 16-18 (E). Pair 16 has an almost median centromere and is easily recognised. Pairs 17 and 18, again very similar to one another, are submetacentric.

Pairs 19-20 (F). The small metacentrics.

Pairs 21-22 and Y (G). The small acrocentrics, of which pairs 21 and 22 may be satellited. The Y is usually the largest of this group, and its arms lie more nearly parallel to one another than do those of the other pairs.

Karyotypes of a normal male and a normal female are shown in Figs. 4 and 5.

The incorporation of tritiated thymidine into the chromosomes can now be studied by autoradiographic techniques. When this thymidine is added to cultures of peripheral blood a short time (c. 3-6 hours) before they are to be harvested, it can be seen that certain chromosomes or chromosome regions replicate later than others. In particular, one of the X

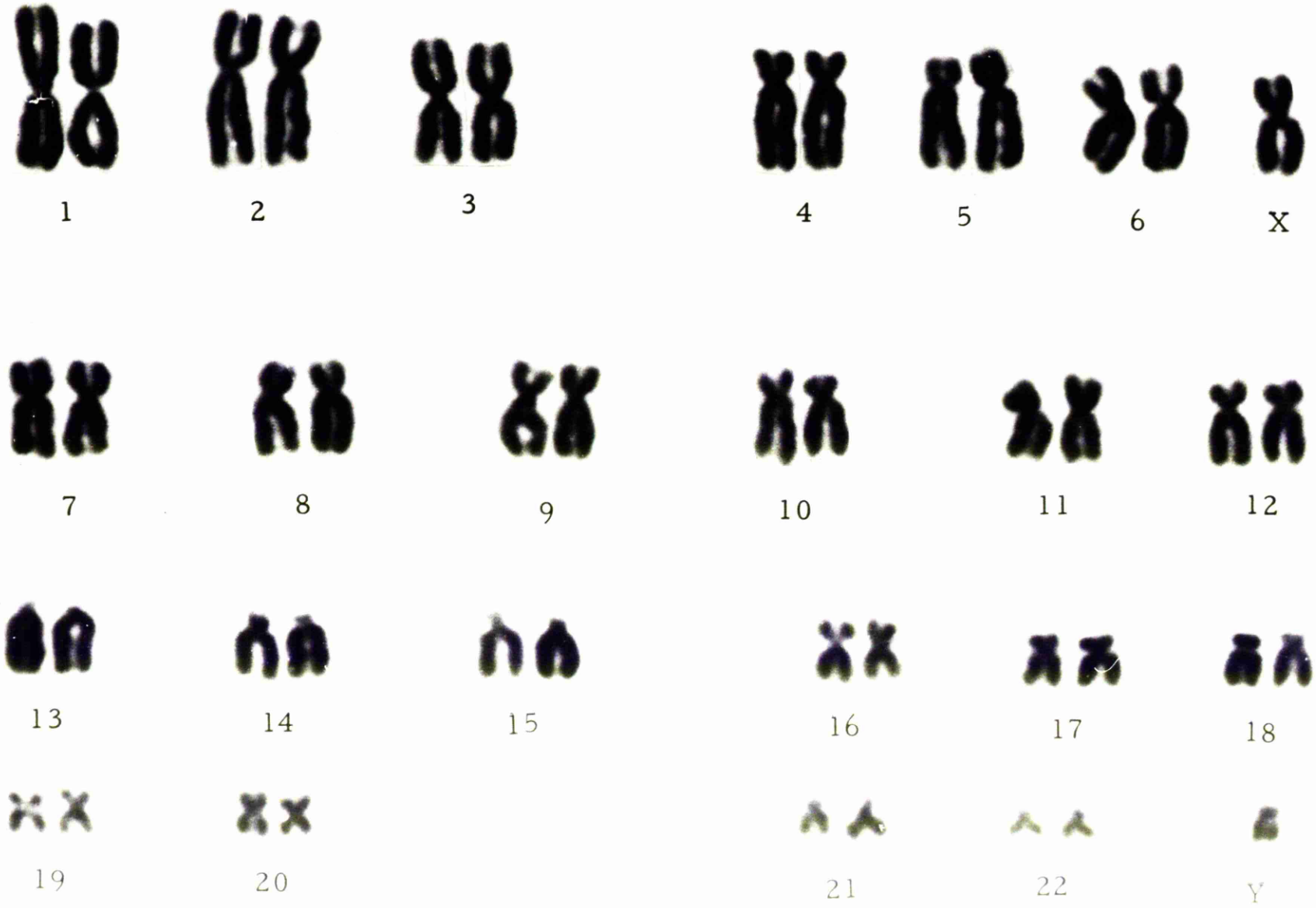


Figure 4. Karyotype of a normal male.

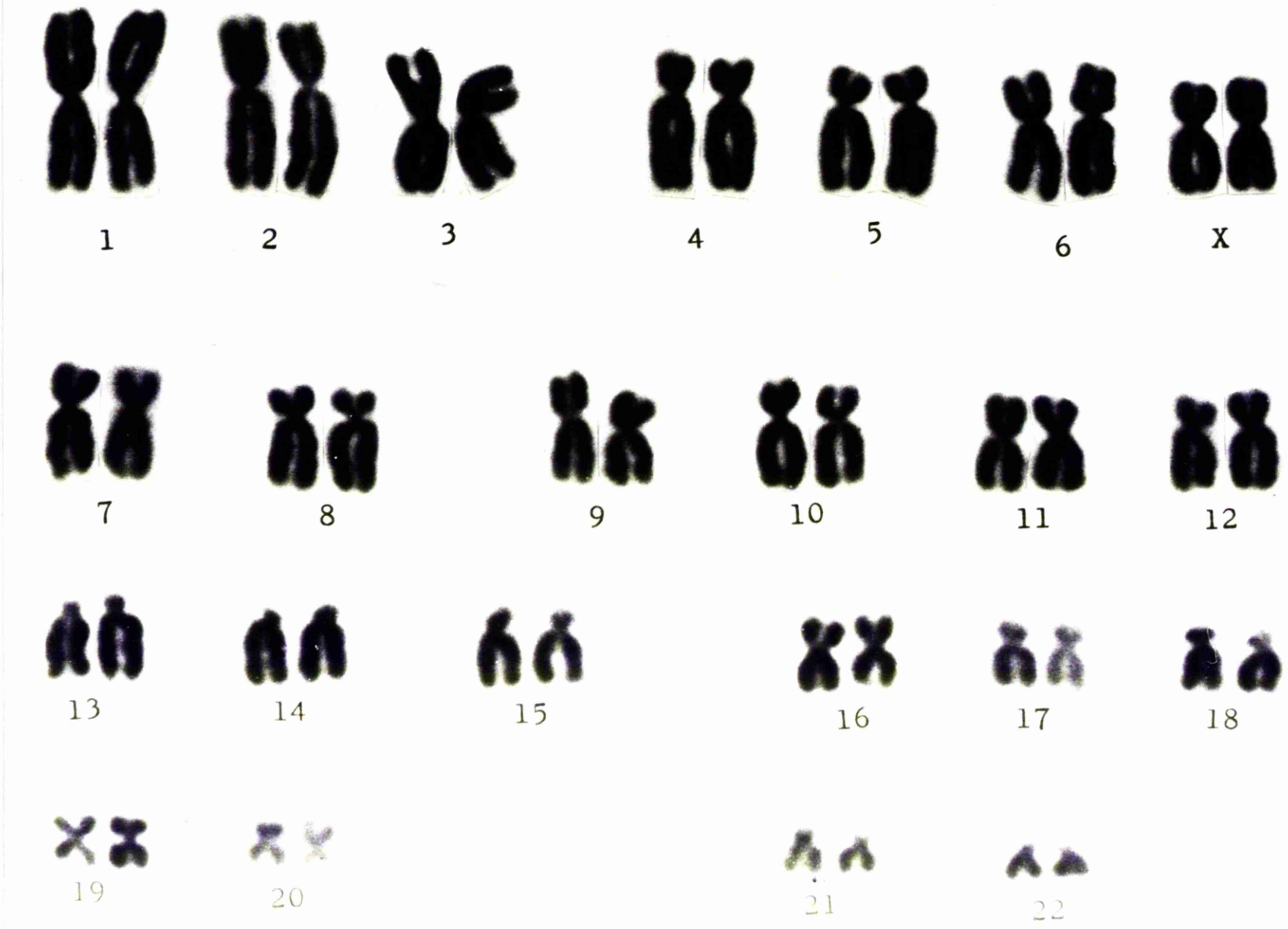


Figure 5. Karyotype of a normal female.

chromosomes in the female can clearly be seen by this method to be very much later in replicating than are the remainder of the chromosomes (Gilbert et al., 1962).

This method is now being extended to help distinguish between the members of a morphologically similar group, e.g. Yunis, Hook and Mayer have investigated the members of group 13-15 (1964 a) in two cases of the D trisomy syndrome, and the members of group 17-18 (1964 b) in a case of E trisomy syndrome. The same group of authors have studied the chromosomes of group 21-22 in several mongols and translocation carriers (1965); German et al. (1964) have used the same techniques to identify the deleted chromosome of group 4-5 in the 'cri du chat' syndrome as one of the pair characterised by relatively early synthesis of DNA along the long arms.

There is also a recent claim by Miller et al. (1963 a and b) that chromosomes tend to occupy the same relative position from cell to cell, but this has not so far been found of value in chromosome identification.

Aneuploid cells in individuals with normal karyotype

(a) Hyperdiploid

Very occasionally a cell is found in which a chromosome has become divided transversely at the centromere, giving apparently two chromosomes with no short arms (telocentric). Such cells have been scored in the present study as structurally abnormal, and not hyperdiploid. Similarly chromosome fragments have been recorded as such, and not scored as chromosomes.

The presence of one or more chromosomes from a different mitosis can generally be detected, because the degree of contraction of chromosomes differs a good deal from cell to cell, depending on the stage of division reached before the addition of colchicine to the cultures.

The remaining hyperdiploid cells are probably genuine products of an error of division, possibly non-disjunction of a diploid cell. In the present study about 1% of cells falls into this category.

(b) Hypodiploid

These are always more frequent than the hyperdiploid, and represent two types of cell, first those genuinely

produced by errors of division such as non-disjunction or anaphase lagging, and secondly those caused by breakage of the cell in handling and subsequent loss of one or more chromosomes. Unfortunately it is not possible to distinguish between these two types since, if a cell is obviously broken, it is not scored in the first place. In the present study about 5% of the cells were of this description. Occasional individuals were discovered with a higher proportion of hypodiploid cells, and yet with no evidence of mosaicism. The reason for this is not obvious as it is not confined to any particular type of patient.

Jacobs et al. (1963) have pointed out that the proportion of aneuploid cells increases with the age of the individual, and in the present study the figures for individuals of normal karyotype are in agreement with this (see Table II). Jacobs et al. claim also that the chromosomes most often affected are, in females, the X, and in males the Y, and that these account for most of the increase in older individuals. In general the figures in the present study seem to confirm this in the case of the Y, but since the majority of the individuals of normal karyotype studied were in the one age group, the figures in the other groups are

too small for reliable conclusions to be drawn. However, the distribution, among the various sub-groups, of the missing chromosomes in 175 cells with 45 chromosomes can be compared with that expected if this loss was random; when this is done it appears that fewer large chromosomes and more small ones are lost than would be expected (see Table III).

TABLE II

Distribution of Counts with Age in 132 Patients
(Male and Female)

Age	No. of Patients	% Cells with Chromosome No. M-1	M	M+1	Total Cells Counted
0-15	46	5	94	1	1917
15-50	71	5	94	1	2888
>50	18	6·5	92	1·5	730

TABLE III

Distribution of Missing Chromosome in 175 M-1 Cells

Chromosome Group	1-3	4-5	6-12	13-15	16-18	19-20	21-22	Y
No. of chromosomes expected	23	15	57	23	23	15	15	4
No. of chromosomes found	15	8	41	25	31	19	29	7

Variation in size of members of chromosome pairs

The two members of a chromosome pair are rarely exactly the same size; this difference is seen even in pairs which can be identified conclusively, such as pairs 1, 2 and 3. Levan and Hsu (1959), in comparing the lengths of the two chromosomes of pair number 1 in 10 cells, found a variation of 0-15%, and Rothfels and Siminovitch (1958) have demonstrated a similar difference between the homologues of pair number 1 in Rhesus monkey. This difference is probably due in part to the limitations of the method of measuring and in part to slight differences in contraction between individual chromosomes.

The Y chromosome in particular seems to show a wide range of variation in size without any phenotypic effect. Cases have been reported in which the Y was almost as large as a group 13-15 (de la Chapelle et al., 1963), and there are several other reports of similar enlargement (Bender and Gooch, 1961; van Wijck, Tijdink and Stolte, 1962; Bishop, Blank and Hunter, 1962). Bender and Gooch saw enlargement in some cells only. Wennstrom and de la Chapelle (1963) concluded that the enlargement they observed was due to

elongation and not to the presence of extra chromosome material, since tritiated thymidine studies showed that the large Ys did not synthesise any more DNA than did control ones. This elongation might be caused by variation in the expression of the secondary constriction in the long arm of the Y.

Chromosome pair 16 can also show more variation in size than the other autosomes (Weber and Miller, 1962).

SECTION III

CHROMOSOME STUDIES IN VARIOUS PATHOLOGICAL CONDITIONS

The object of this study was to find out if there were any chromosome abnormalities which could be associated with pathological conditions in patients. The conditions fell into four categories:

(1) conditions known to be genetically inherited,

(2) other syndromes,

(3) multiple congenital abnormalities,

(4) diseases of the blood.

(1) Conditions known to be genetically determined

The chromosome counts are shown in Table IV.

Peutz-Jeghers Syndrome

Several cases of this rare syndrome are described by Jeghers, McKusick and Katz (1949). The two main features are the presence of distinctive melanin spots on the buccal mucosa and lips - face and digits may also be involved - and polyposis of the small intestine. The disease may be familial, and shows simple Mendelian dominance.

The chromosomes of a boy with this condition (No. 28) were examined, and were apparently normal.

Laurence-Moon-Biedl Syndrome

This is a hereditary syndrome involving obesity, hypogenitalism, retinitis, mental deficiency, skull defects and syndactyly. The chromosomes of one man with this syndrome were studied by the author (No. 76). The number of cells available for study was small, but the chromosomes were apparently normal. The same result has been obtained by Miller and Jacobsen (1962) on two affected brothers.

Myotonica dystrophia

This is a hereditary syndrome of progressive muscular degeneration; there is a marked difference in both age of onset and severity of symptoms between various generations. It is generally believed to be inherited as an autosomal dominant, and Penrose (1947) has suggested a modifying factor to account for the variation. Sex chromatin has been shown to be normal by Marshall and Thomas (1958).

Fitzgerald and Caughey (1962) examined the chromosomes of seven patients with the disease. In five of these they report finding a small proportion of cells in which there was an additional chromosome similar to pairs 21 and 22, and which they suggest may be a supernumerary. The three cases which show this chromosome with the highest frequency are those in

which the disease was most severe. The findings of the author in two patients with this condition (Nos. 107, 268) are not in agreement with this observation. The second case studied was a severe one (patient died aged 37 years), but no cells with 47 chromosomes were found. The first case had one such cell but this was thought to be due to a broken chromosome, and there was certainly no additional chromosome of the appropriate size present. However, the proportion of cells observed by Fitzgerald to have the supernumerary chromosome was small. The highest frequencies were in two patients, the first of whom had 7 such cells out of a total of 65 cells counted, and the second had 3 such cells out of 43 cells counted. The remaining three cases each had only one such cell out of a total of about 40 cells counted. The number of cells counted from the two patients in the present study was small, 18 in the first case and 34 in the second, so the presence of a supernumerary chromosome cannot be entirely ruled out. On the other hand, the evidence for the existence of supernumerary chromosomes in man is at present unconvincing. Their presence was suggested by Stern (1959) to account for the three chromosome numbers reported by Kodani (1957), but Kodani's work has never been confirmed despite extensive studies on Japanese patients by Makino.

Huntingdon's Chorea

This is a serious, incurable nervous disease, usually developing rather late in life, and thought to be due to a dominant allele. The chromosomes of a woman (No. 259) with this disease were studied and were apparently normal.

Marfan's Syndrome

This is a congenital and hereditary disorder of connective tissue, the patients being abnormally tall and thin, with arachnodactyly and bilateral displacement of the lens of the eye, and frequently aortic aneurysm of a special type. The chromosomes of two patients with this syndrome were investigated (Nos. 292, 406).

The first chromosome observations on patients with this syndrome showed no obvious chromosomal abnormality (Tjio, Puck and Robinson, 1959; Ford, 1960); but two anomalies have since been claimed. Tjio, Puck and Robinson (1960) claimed greatly enlarged satellites in two patients with familial inheritance of the disease, in one instance in a chromosome 21 and in the other a chromosome 13. The fact that the chromosomes involved are different in the two cases makes it unlikely that this enlargement of the satellites is responsible for the disease, especially in view of the fact that the same phenomenon

has been observed in many individuals who do not have Marfan's syndrome (Cooper and Hirschhorn, 1962; Ellis and Penrose, 1961; present study, section on mongolism). Handmaker (1963) has examined the chromosomes of eight patients with Marfan's syndrome without finding any whose satellites were enlarged to a greater extent than those of their unaffected relatives.

Neither of the patients whose chromosomes were examined here showed any extraordinary enlargement of the satellites; the first patient (No. 292) had a Y chromosome of greater than average length (see Fig. 6), but again this phenomenon has been observed in many individuals without Marfan's syndrome (Bender and Gooch, 1961) and is probably without relation to the disease. An unusually large Y chromosome in a case of Marfan's was described by Kallen and Levan (1962) in the paper in which they report that in four patients with this disease the chromosomes of group 21-22 were shorter than those of normal controls. This difference was not obvious on normal microscopic examination, but only became evident on analysis of the lengths of the chromosomes. They suggest that this is a change in the phenotype of the chromosomes brought about by a genetic modification. Since the difference in length was not immediately obvious, however, it is possible that a

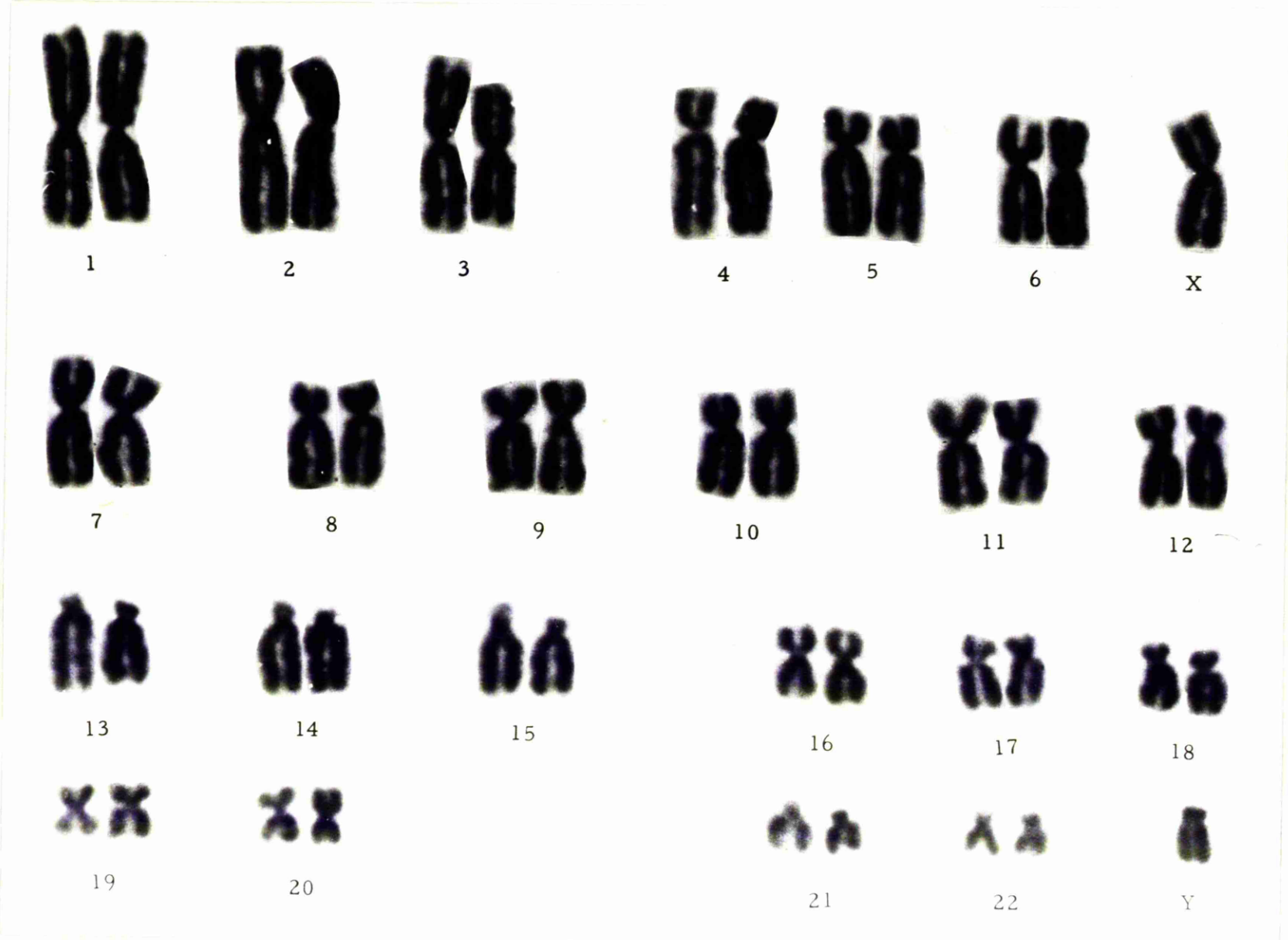


Figure 6. Karyotype from patient No. 292, a case of Marfan's syndrome, showing the presence of a large Y chromosome.

wider range exists in the general population than is at present realised. On the whole, it does not appear that a consistent chromosome abnormality is connected with Marfan's syndrome.

Breast carcinoma

Chromosome examination was performed on a mother and her two daughters, all of whom showed a strikingly similar type of breast carcinoma which it was believed might be genetically controlled. Cultures from one of the daughters failed to grow, but in those of the mother and remaining daughter no abnormality was observed (Nos. 346, 347).

TABLE IV

Chromosome Counts in Patients with Genetically Determined Conditions

Ref. No.	Disease	Sex	Cells with Chromosome No. 45	46	47	Total
28	Peutz-Jeghers	M	1	14	1	16
76	Laurence-Moon-Biedl	M	-	10	-	10
107	Myotonica	M	2	15	1	18
268	Dystrophia	M	4	30	-	34
259	Huntingdon's Chorea	F	4	33	2	39
292	Marfan	M	2	58	-	60
406	Syndrome	M	3	47	1	51
346	Breast	F	6	35	-	41
347	Carcinoma	F	3	33	-	36

(2) Other Syndromes

The chromosome counts are shown in Table V.

Frohlich's Syndrome

This is a condition of adiposogenital dystrophy, due to hypopituitarism. The chromosomes of the man investigated in this study (No. 64) were apparently normal.

Cretinism

In this condition there is a congenital lack of thyroid secretion which leads to arrested physical and mental development. One girl, whose mother was also affected, was studied by the author and had apparently normal chromosomes (No. 132).

Sturge-Weber Syndrome

This is a syndrome of gross mental retardation and hyperkinesis, seizures, and congenital cutaneous angiomatous naevi involving face and other areas of the body. The chromosomes of a boy with this syndrome were investigated in the present study (No. 81).

Hayward and Bower (1960) reported chromosome studies from the bone marrow of a 3 year old boy with this syndrome. They found 47 chromosomes to be present, and claimed trisomy for a

member of group 21-22. This was identified as 22 by the absence of satellites on the short arms of the extra chromosome. Although it is now known that all members of this group can be satellited, the extra chromosome is unlikely to have been a 21 since none of the features of mongolism were present. Later, however, the same authors reported (Hayward and Bower, 1961) on a further 7 patients, in all of whom the chromosomes were normal, and this result has been confirmed by other authors (Lehman and Forssman, 1960 - two patients; Gustavson and Hook, 1961 - two patients; Hall, 1961 - three patients). The patient studied by this author also had normal chromosomes. In their later paper Hayward and Bower suggest that the 47th chromosome is a chance association of events, or perhaps a supernumerary. It has also been suggested (Lancet L.A., 1960) that it might be an additional Y, which is known to have little phenotypic effect (Sandberg et al., 1961).

Patau and his co-workers (1961) also describe three patients with Sturge-Weber syndrome; in two of these the karyotype was apparently normal but the third had a small segment of chromosome material translocated on to the short arms of a member of the 13-15 group. They postulate that

this represents partial trisomy of an unknown donor chromosome, and claim that such a condition is always present in the Sturge-Weber syndrome, though not always detectable microscopically. They thus explain the 47th chromosome in Hayward and Bower's first case as a translocation chromosome. There is no evidence to support this view at present, nor the further hypothesis of Patau that partial trisomy underlies many cases of congenital abnormalities. However, it cannot be denied that there are many chromosomes in the human complement in which a small structural alteration would be overlooked by the methods at present available.

Typus degenerativus Amstelodamensis (de Lange's Syndrome)

Five patients are described by Jervis and Stimson (1963): the symptoms are summarised as mental retardation and a cluster of minor physical malformations including small stature and head circumference, low forehead with heavy confluent eyebrows, depressed root of nose and flaring nostrils, low set ears, clinodactyly of 5th finger, low placed thumb, webbing of 2nd and 3rd toes, and hypertrichosis.

Jervis also studied the chromosomes of his patients and reported a normal karyotype except for the fact that four out of the five cases had a percentage of cells containing a small

fragment. The significance of these cells was not known. The chromosomes of two patients with this syndrome were examined by Laurence and Ishmael (1963) and found to be normal, and in a further case reported by Hienz (1963) the same result was obtained.

The chromosomes of three affected boys were examined in the present study, and again a normal karyotype was found (Nos. 371, 368, 369). The Y chromosome was somewhat larger than usual in one of these cases: this is probably a normal variation unconnected with the disease, as was the case with Marfan's syndrome. In this same patient (No. 371) there was a higher percentage of cells with 45 chromosomes than usual, but there was no consistency in the missing member.

TABLE V

Chromosome Counts in Patients with Other Syndromes

Ref. No.	Condition	Sex	Cells with Chromosome No. 45	46	47	Total
64	Frohlich	M	3	23	-	26
132	Cretin	F	2	45	-	47
81	Sturge-Weber	M	3	25	-	28
371	Typus	M	8	56	-	64
368	Degenerativus	M	3	45	-	48
369	Amstelodamensis	M	-	50	-	50

(3) Other Congenital Abnormalities

The chromosome counts are shown in Tables VI and VII.

Anencephaly

This is a developmental abnormality with absence of neural tissue in the cranium.

Sex chromatin studies were done by Bearn (1959) and Polani (1959) and the nuclear sex was found to correspond to the phenotypic sex. Harnden et al. (1959) showed the chromosomes of two male and two female anencephalics to be normal.

In this study chromosome investigations were performed on three couples, each of whom had had two anencephalic foetuses, and also on one mother who had one hydrocephalic, two miscarriages and one anencephalic. In all these seven individuals the karyotype was within the normal limits.

TABLE VI

Parents of Anencephalic Foetuses

Ref. No.	Sex	Cells with Chromosome No. 45	46	47	Total
131)	M	3	24	-	27
138)	F	3	34	-	37
376)	M	2	44	-	46
377)	F	-	48	-	48
431)	F	-	-	-	-
432)	M	2	58	2	62
304	F	1	30	-	31

Multiple Congenital Abnormalities

There have been several instances where congenital abnormalities have recently been discovered to be associated with irregularities of the chromosome complement. Both the chromosomes involved and the type of abnormalities present have varied from case to case, making it difficult to see the relationship between the irregular chromosome pattern and the pathological condition of the patient. Chromosome abnormalities reported have included ring chromosomes (Genest, Leclerc and Auger, 1963; Wang et al., 1962; Bain and Gauld, 1963; Lindsten and Tillinger, 1963; Turner et al., 1962), deletion (Lejeune et al., 1963), translocation (Rohde and Catz, 1964; Vislie et al., 1962), and diploid-triploid mosaicism (Ellis et al., 1963; Ferrier et al., 1964). On the other hand many cases of congenital abnormalities studied have proved to have a normal chromosome complement (Rohde, 1962; Klevit, Loftus and Mellman, 1962; Hern, Hamm and Robertson, 1963; Pfeiffer, 1964). The chromosomes of eight individuals, selected because of various congenital abnormalities, in the present study (see Table VII) were also normal.

Only in three instances - the D and E trisomy syndromes, and the 'cri du chat' syndrome - has a distinct pattern emerged.

The D syndrome was first described by Patau and his co-workers in 1960, and involves trisomy of a member of group 13-15. This is associated with cerebral defect, apparent anophthalmia, cleft palate, hare lip, simian creases, trigger thumbs, polydactyly, and heart defect. Children born with this condition do not generally survive for long.

'Cri du Chat' Syndrome. Three cases were reported by Lejeune et al. (1963) and several others are now known. The name comes from the peculiar meowing cry characteristic of children with this syndrome. The other symptoms include severe mental retardation, microcephaly, somatic dwarfism, and transverse palmar creases. The chromosome abnormality involved here is a deletion of part of the short arms of a chromosome of group 4-5.

The E syndrome was first described by Edwards and his group in 1960. It involves trisomy for a chromosome of group 17-18, now generally recognised to be a number 18, and the main abnormalities are odd-shaped skull, low-set and malformed ears, triangular mouth and receding chin, short stubby fingers and toes with short nails, webbing of toes, congenital heart condition and mental retardation.

In the present study, the chromosomes of one patient suspected of having this syndrome were examined. The infant,

a slightly premature girl born at maternal age 30 years, weighed 2480 gm. and measured 45 cm. at birth. She failed to thrive, and died after 7 days. Her hands showed clawing, lateral displacement of 4th and 5th fingers and small fingernails. Both feet had syndactyly of 2nd and 3rd toes. There was an abnormally shaped skull, the posterior parietal diameter being wider than the frontal. Ears were low-set, and the eyes were prominent, with a membrane over the cornea. There was bilateral complete hare lip, and cleft palate.

The heart filled almost half the thoracic cavity; there was a right-sided hypoplasia with complete pulmonary stenosis and absence of the tricuspid valve. The septum of the foramen ovale was fenestrated. Both atria were grossly distended; the right ventricle was a narrow slit and the left ventricle was grossly enlarged. Mitral valve showed no abnormality.

A Meckel's diverticulum was present. Histological examination of ovary showed extremely scanty primordial follicles.

The chromosome preparations were rather disappointing in quality but could not be repeated as the infant died. The modal number was 47 (see Table VII), and careful analysis of

five cells clearly showed that the extra chromosome was in group 17-18 (see Fig. 7).

TABLE VII

Chromosome Counts in Patients with Congenital Abnormalities

Ref. No.	Condition	Sex	Cells with Chromosome No. 45	46	47	48	Total
35	Congenital liver dysplasia	M	2	16	-	-	18
391	Abnormal centres ossification. Malformed low-set ears. Talipes. Large nose. No scrotum. Chr -ve	M	1	47	-	-	48
410	Part of sternum missing. Hypospadias.	M	5	45	-	-	50
411	Multiple congenital abnormalities. Dwarfism.	F	1	49	-	-	50
384	Congenital heart condition. Haemolytic anaemia.	F	2	47	1	-	50
265	Malformed head and eyes. Very long fingers. Underdeveloped scrotum.	M	-	19	-	-	19
354	Cleft palate, small chin. Talipes.	F	1	34	-	-	35
337	Abnormal facies with microphthalmia. Micrognathia ++. Low-set ears. Very short neck.	F	-	11	-	-	11
331	Trisomy 17/18. See text	F	-	2	22	-	24

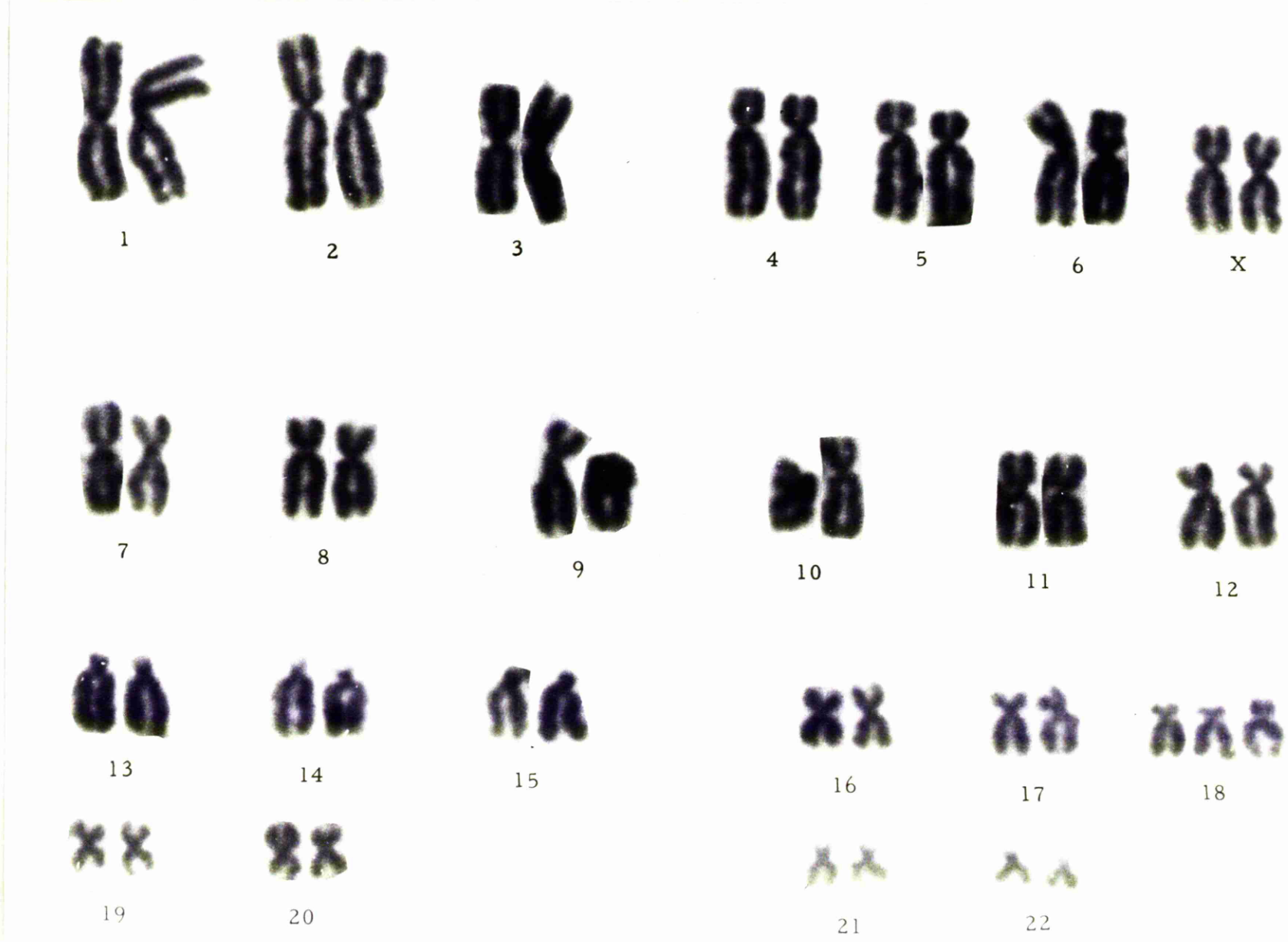


Figure 7. Karyotype of a cell from patient No. 331, showing trisomy for a chromosome of group 17-18.

(4) Chromosomes in Diseases of the Blood

The chromosome counts are shown in Table VIII.

Many cytogenetic studies have been reported on the various types of leukaemia. This is a field where observations must be made on a large scale - such as those carried out by the Edinburgh group - in order to produce significant results, and is quite beyond the scope of the present study.

In the present work the presence of the Philadelphia (Ph^1) chromosome (Nowell and Hungerford, 1960) has been confirmed in two cases of chronic myeloid leukaemia (see Fig. 8). The chromosomes of 14 patients with myeloproliferative syndrome have been studied. No Ph^1 was observed in any of the 14 cases, nor was there any obvious deviation from the normal karyotype. This is in agreement with the findings of other authors; Sandberg et al. (1962) found no Ph^1 in twelve patients who had developed a leukaemia-like picture following myelofibrosis or polycythaemia vera. Nowell and Hungerford (1962) found normal karyotypes in their patients with myeloproliferative disease, with the exception of two cases of myelofibrosis who had developed granulocytic leukaemia, and one of polycythaemia vera who had had extensive ^{32}P therapy.

Both these groups of authors emphasise the difference in chromosome pattern between cells of marrow treated directly,

Figure 8. Mitotic figure from a patient with chronic myeloid leukaemia, showing Ph^1 chromosome (arrowed).

and cells of cultured blood. Blood cultures have a far higher proportion of normal cells, probably owing to the inability of the unbalanced cell-lines to divide under culture conditions.

TABLE VIII

11 Cases, Myelofibrosis, 2 Cases Polycythaemia Vera

Ref. No.	Cells with Chromosome No. 45	46	47	Total	Ph Present *	Ph Absent
143	-	18	1	19	-	9
154	2	8	-	10	-	9
155	2	27	-	29	-	18
176	2	33	-	35	-	21
213	1	30	1	32	-	16
218	-	7	-	7	-	6
233	4	51	-	55	-	39
236	-	22	-	22	-	11
330	4	38	-	42	-	18
361	-	31	-	31	-	21
378	2	32	-	34	-	21
219	1	30	-	31	-	17
258	1	34	2	37	-	22

* Cells were not scored for the presence of the Philadelphia chromosome unless all members of group 21-22 could be seen perfectly.

Chromosome studies were carried out on a patient with a diagnosis of aleukaemic chronic myeloid leukaemia (No. 448).

This patient, a 68 year old male, had an enlarged spleen. Examination of blood revealed Hb 72% white cell count 6,100/c.mm., platelets 180,000 per c.mm. The differential proportions were normal; no abnormal forms were seen. Leukocyte alkaline phosphatase activity was very low.

Sternal marrow examination showed gross leukopoietic hyperplasia, and an increase in the proportion of myelocytes. Thus clinical findings were consistent with, and bone marrow appearances typical of, chronic myeloid leukaemia and a diagnosis of aleukaemic chronic myeloid leukaemia was made.

Bone marrow examination was repeated 3 months later and revealed a gross increase in the myeloid series with maturation arrest at the myelocyte stage. An increase in the number of megakaryocytes was noted. Alkaline phosphatase activity in the neutrophil series was very low. In blood films occasional myelocytes and myeloblasts have been seen but the total white cell count and the platelet count have remained within the normal range.

Chromosome studies were performed on bone marrow material on both these occasions; the counts are shown in Table IX. (See also Fig. 9.)

The cells were cultured for 3 hours at 37°C with colchicine before examination. On both occasions the modal number was 45; there were only 4 chromosomes present in group 21-22-Y and the karyotype was interpreted as XO. Ph^1 was absent. Cultures prepared in the usual manner from peripheral blood showed a normal diploid karyotype - again Ph^1 negative. The cells seen dividing in these phytohaemagglutinin-treated blood cultures are thought to come from the lymphocytic series (McKinney et al., 1962), and so it is not uncommon for such cultures from patients with chronic myeloid leukaemia to contain a proportion of cells without the Ph^1 chromosome.

This patient combines two cytogenetical features which have previously been observed singly in connection with chronic myeloid leukaemia.

A small proportion of patients with the typical findings of this disease have been shown to be Ph^1 negative (Tough et al., 1963; Speed and Lawler, 1964). Tough has suggested that this sub-group may have a different mechanism of origin from the majority. The presence of an XO cell-line has likewise been described before in chronic myeloid leukaemia. Tough et al. (1963) report two cases of males with an XO cell-line in the bone marrow, one of which has also a diploid

Figure 9. Karyotype of a 45/XO cell from the bone marrow of patient No. 448. No Ph^1 chromosome is present.

cell-line present, and Speed and Lawler also described two males with 45 chromosomes in the cells of the bone marrow where only 4 small acrocentrics were present. In these two latter cases the disease has had a very chronic course - the patients being on a maintenance dose of busulphan for 8 years. All these four patients, however, had the Ph^1 chromosome present.

A male with chronic myeloid leukaemia was reported by Atkin and Taylor (1962) to have in blood cultures a cell-line of Ph^1 negative diploid cells and a Ph^1 positive line with 45 chromosomes, only 4 small acrocentrics being present.

Since aleukaemic myeloid leukaemia is a very rare condition, the finding of atypical chromosomes in this case is of interest. However, since other cases of chronic myeloid leukaemia have been reported with various other chromosome anomalies, it may be purely accidental and non-specific.

TABLE IX

Chromosome Counts in Patient No. 448

Material	No. of Cells with Chromosome No. 44	45	46	47
Bone Marrow (1)	4	35	-	-
Bone Marrow (2)	2	21	1	-
Peripheral Blood	-	2	47	2

SECTION IV

CHROMOSOME STUDIES IN MONGOLISM

Langdon-Down (1866) attempted to explain various clinical forms of mental deficiency as a regression to other races. His argument is not accepted today, but the group which, because of their superficial resemblance to the Mongolian race, he called Mongol, is still most commonly known by this name.

The group of mental defectives which he described under this name has no one common diagnostic feature, although they bear a striking resemblance to one another, especially in childhood. The disease is very difficult to define; it is a complex of physical abnormalities, mostly developmental defects, associated with mental defect. The chief features used by two authorities in diagnosing the disease are given in Table X. Both agree that no mongol shows all the signs involved, but that any defective with four or more of them is probably a mongol. The diagnosis can be especially difficult to make in the newborn, since some features such as the furrowed tongue and peculiarity of the iris are not seen in infants. Mongols have been the subject of extensive study,

and deviations from normal in many tissues have been reported.

TABLE X

Symptoms of Mongolism

Penrose 1933	Øster 1953
1) I.Q. 15-29	1) 4 finger line
2) cephalic index over 0·83	2) short crooked 5th finger
3) epicanthus	3) short broad hands
4) furrowed tongue	4) hyperflexibility
5) conjunctivitis	5) oblique palpebral fissures
6) 4 finger line	6) epicanthus
7) one flexion furrow of 5th finger	7) furrowed tongue
	8) abnormal sets of teeth
	9) high narrow palate
	10) flat occiput

There have been several estimates of the incidence of mongolism among newborn infants, e.g. Jenkins (1933), 1 in 636; Carter and McCarthy (1951), 1 in 660. When these figures are compared with those obtained by Penrose (1949) for school-children - an incidence of about 1 in 3,000 - the fact is emphasised that many mongols do not survive the first few years of life.

Cytology of Regular Mongols

This was the first condition in man to be shown to be due to a chromosomal anomaly. In 1959, several authors (Lejeune, Gauthier and Turpin; Jacobs et al.; Ford et al.; Böök) reported that mongolism was associated with the presence of 47 chromosomes, the extra one being in the group of the smallest acrocentrics. It was decided that the extra chromosome was most likely to be an additional 21, because of the close similarity in size. In addition it was observed to have satellites, which at that time were thought to be confined in this group to pair 21. This is still the generally accepted view, although there is now evidence that all the chromosomes in group 21-22 can be satellited.

This trisomic condition is thought to result from non-disjunction involving chromosome 21 during gametogenesis in one of the parents, leading to the production of a gamete with two 21s. When fertilised by a normal gamete, trisomy results. Since the incidence of mongolism is known to rise with increasing maternal age, it seems likely that such non-disjunction usually occurs in the mother.

Maternal Age

It was early noticed that mongols were frequently born

to older mothers, and were often at the end of a large sibship. This could have been due to any or all of three closely related factors - maternal age, paternal age, and order of birth. This problem has been the subject of extensive research by Penrose (1933, 1934-35, 1934), who has clearly shown that the important factor is the age of the mother. It is now agreed that the risk of a mongol birth increases steadily with increasing maternal age.

However, Penrose (1951) points out that the distribution of the incidence of mongol births according to maternal age shows two maxima - one at c. 27 years and the other at c. 42 years, and that there are two occasions when the age of a mother at the birth of a mongol child is significantly lower than the average for such births. These are, in the first place, when there are two or more affected individuals in a sibship, and in the second place when there are two or more affected members in a family, though in a more distant relationship, and particularly when transmission is through the maternal line.

This discovery led to extensive investigation of such affected families. A study in 1960 by Polani et al. brought to light a different mechanism in some cases. Examining the

chromosomes of mongols born to young mothers, he discovered one girl with only 46 chromosomes: there were only four in group 21-22 instead of the expected five, only five in group 13-15 instead of six, and an additional one in group 6-12. This was interpreted as being the result of a reciprocal translocation between an acrocentric of group 21-22 and an acrocentric of group 13-15; the other, smaller, product had been lost, but the child still had the greater part of both chromosomes and was in effect trisomic for chromosome 21.

Since then it has been shown that such a chromosome can be inherited from a parent who is himself, or more often herself, phenotypically normal but a carrier of the translocation; such transmission has been traced through several generations in some families. It is also known for two chromosomes of group 21-22 to take part in such a translocation, the product here resembling a member of group 19-20.

Present Investigation and Results

The present investigation has been concentrated on families which might be in the situation just described. Chromosomes were studied in the following series of individuals

where the mother was under 30 years of age at the birth of her mongol child:-

5 cases, mongol child and both parents are shown in Table XI

6 cases, mongol child and mother only are shown in Table XII

9 cases, both parents but not child, are shown in Table XIII.

TABLE XI

Both Parents and Mongol Child

Ref. No.	Cells with Chromosome No. 45	46	47	48	Total
301	2	41	-	-	43
300	-	31	-	-	31
299 (M)	-	1	49	-	50
317	3	47	-	-	50
319	7	47	-	-	54
320 (M)	-	2	37	-	39
324	6	44	-	-	50
325	4	49	2	-	55
326 (M)	-	2	46	-	48
383	2	48	-	-	50
382	1	50	-	-	51
381 (M)	3	34	1	-	38 *
421	2	48	-	-	50
420	2	42	1	-	45
419 (M)	-	1	50	1	52

M = mongol child * translocation mongol

TABLE XII

Mother and Mongol Child

Ref. No.	Cells with Chromosome No. 45	46	47	48	Total
256	1	13	1	--	15
257	--	--	--	--	--
303	2	43	--	--	45
302	--	5	45	--	50
380	8	44	--	--	52
379	--	1	51	--	52
385	--	50	--	--	50
386	--	6	44	--	50
393	3	46	--	--	49
392	--	2	47	--	49
417	1	52	--	--	53
418	--	4	58	--	62

TABLE XIII

Parents of Mongol Child

Ref. No.	Cells with Chromosome No. 45	46	47	48	Total
321	3	47	-	-	50
322	2	46	2	-	50
327	2	37	-	-	39
328	1	28	1	-	30
332	1	45	-	-	46
333	5	45	-	-	50
334	1	41	-	-	42
336	3	45	-	-	48
338	5	29	1	-	35
339	-	32	1	-	33
343	1	31	1	-	33
344	1	49	1	-	51
351	3	39	-	-	42
353	1	31	-	-	32
373	2	48	-	-	50
374	2	48	-	-	50
366*	14	150	14	-	178
367	3	47	-	-	50

* mosaic mother - see text.

With one exception, the mongols were found to have a chromosome complement of 47, and trisomy 21 (see Figs. 10 and 11). The one exception (No. 381) had 46 chromosomes, and the karyotype was interpreted as representing a translocation between two members of group 21-22 (see Fig. 12). The chromosome complement of both parents was normal.

Again, with one exception, the chromosomes of the parents were normal. Aneuploid cells were carefully checked to eliminate any possibility of mosaicism. One mother and her mongol baby were found to have enlarged satellites on a member of group 13-15, but, as mentioned in connection with Marfan's syndrome, this is probably a normal variation.

The exceptional parent, No. 366, was a mother. In her cells with 45 chromosomes, the missing member was not consistent, but in 12 out of the 14 cells with 47 chromosomes the extra element was interpreted as a 21. Cultures were prepared on two separate occasions with the same result, so that it is unlikely that an event occurring during cell culture could account for this finding. It was concluded that the patient was a mosaic with a normal stem-line and also a very low proportion of a 47-chromosome cell-line with trisomy for No. 21. She herself had none of the features of

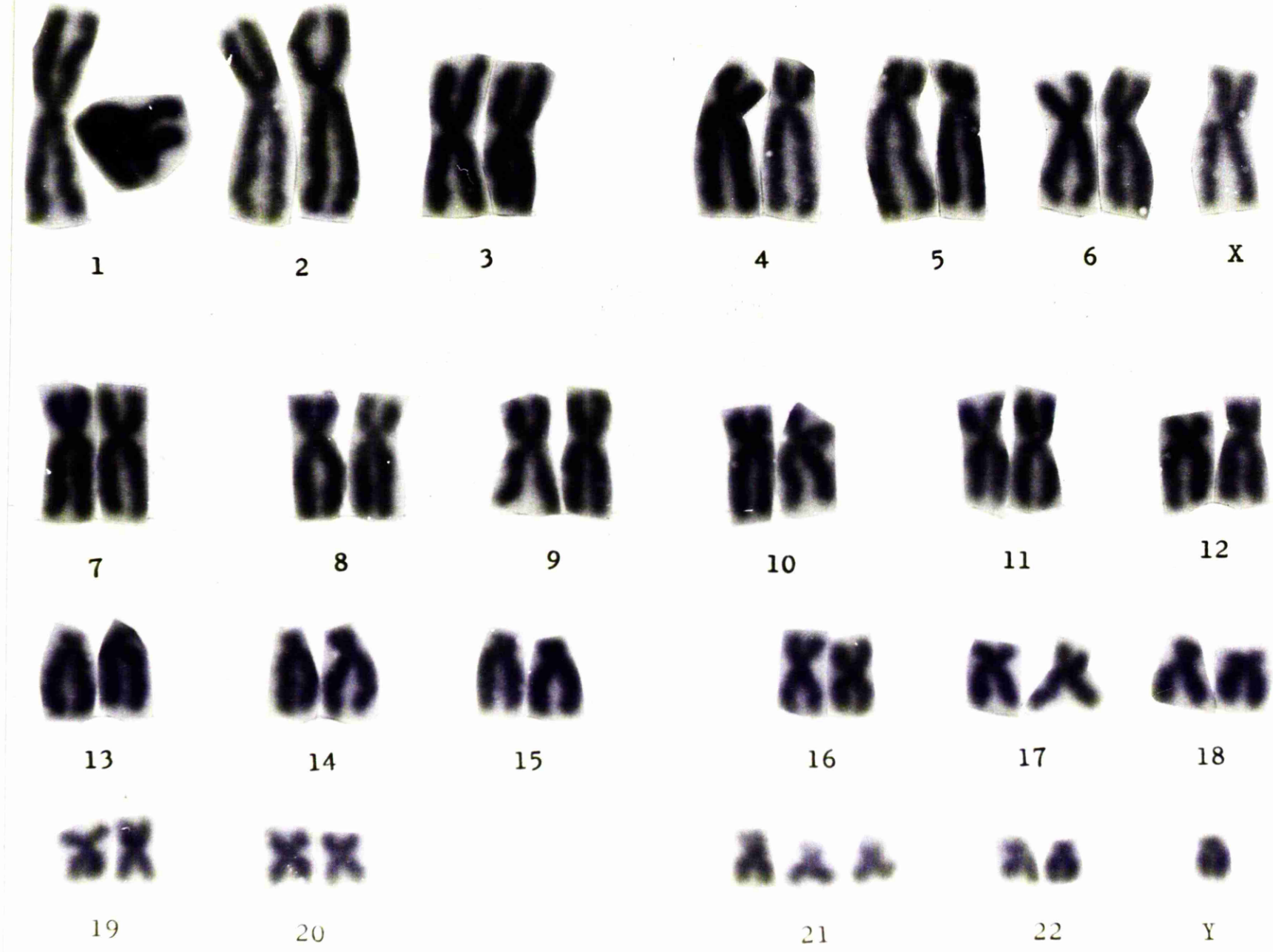


Figure 10. Karyotype of a male mongol.

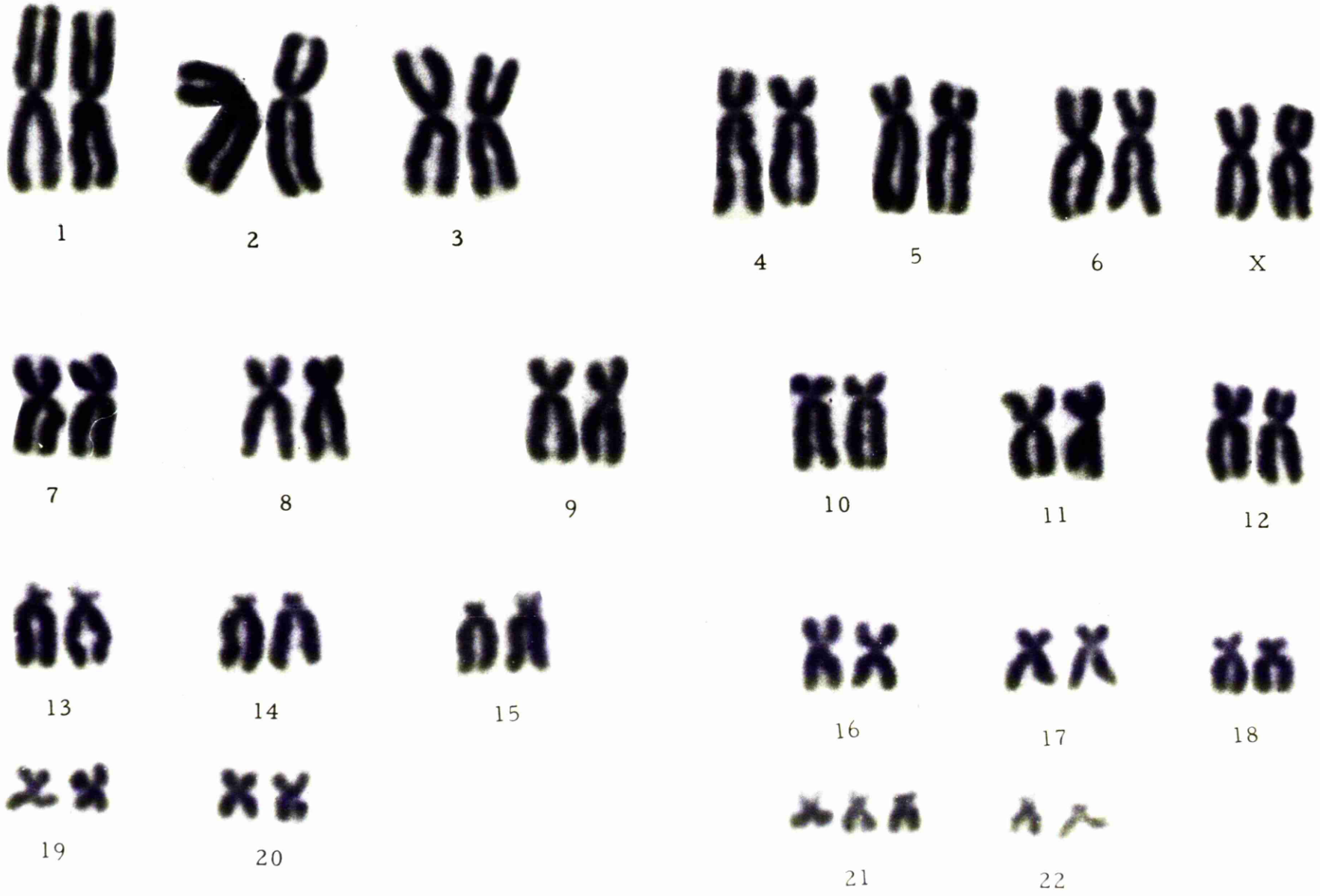
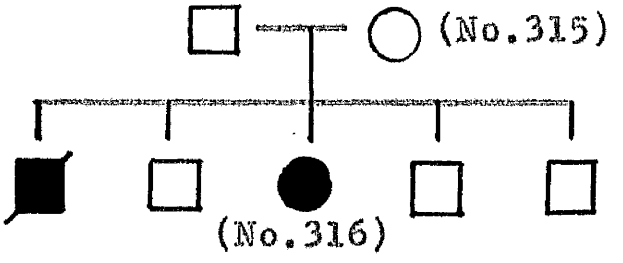


Figure 11. Karyotype of a female mongol.

mongolism. Her husband and mother had normal karyotypes.

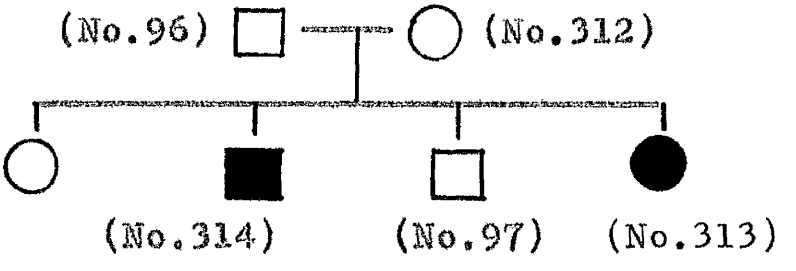
Two families with two affected sibs were investigated. The chromosome counts are shown in Table XIV.

Family C



The mother had normal chromosomes; the surviving mongol (born at maternal age 34 years) was trisomic for chromosome 21.

Family S



The parents and the normal sib studied had unexceptional chromosome complements; both mongols (born at maternal ages 35 and 41 years) were trisomic for chromosome 21.

Chromosomes were also studied in a father and his two daughters (Nos. 307-309), one of whom was a mongol. The father had an uncle, not born late in maternal life, who was also a mongol. Again the mongol was trisomic for chromosome 21 and the other two were normal.

Two mongols with higher I.Q.s than usual were studied chromosomally, but both proved to have an unmixed 47-chromosome complement with trisomy 21.

In five instances where there was a genuine doubt about the diagnosis of mongolism, chromosome studies were performed (Table XIV). Three of these proved to have the usual mongol complement, while the other two had normal chromosomes.

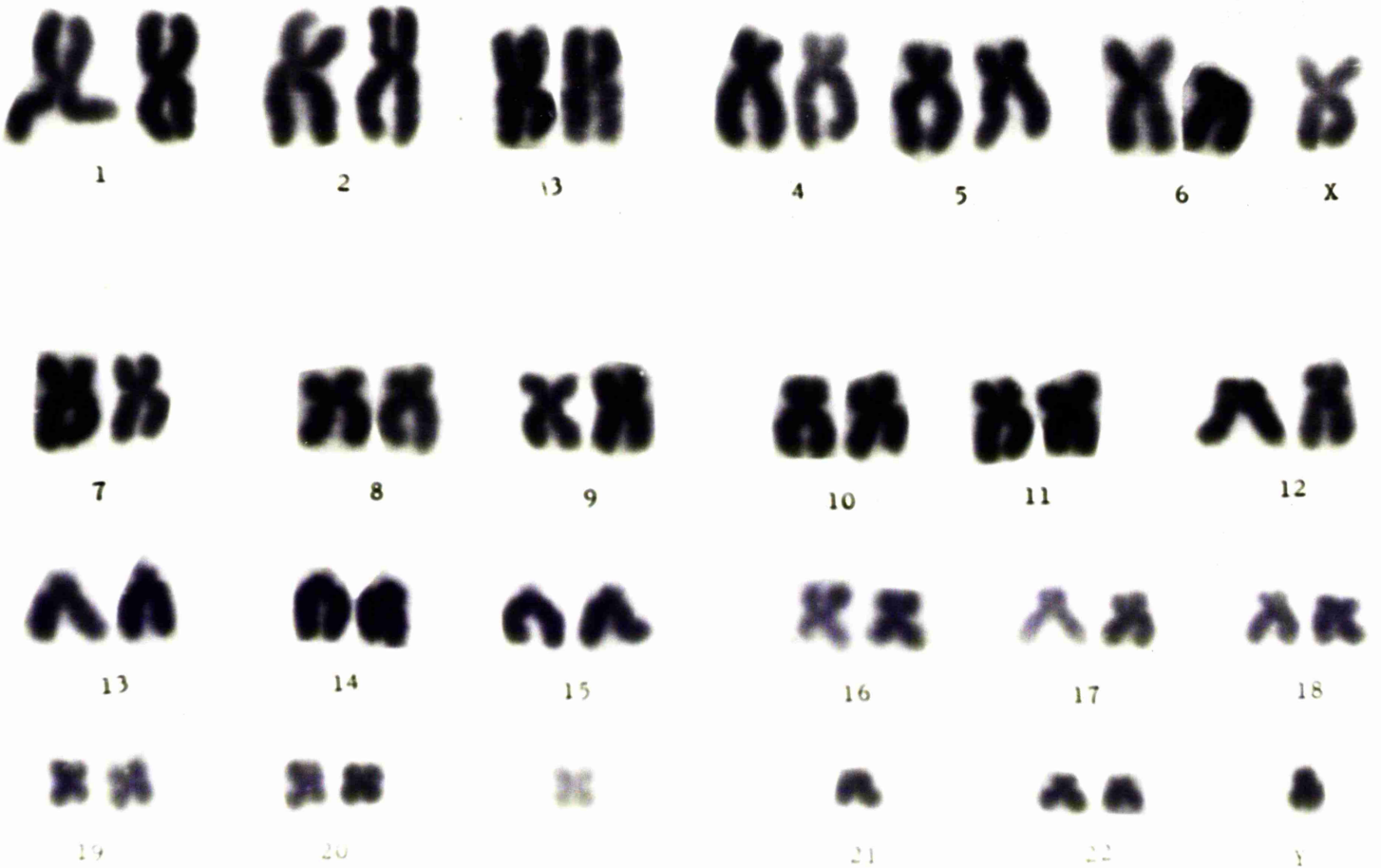


Figure 12. Karyotype of a male mongol with a translocation between two chromosomes of group 21-22.

TABLE XIV

Various Mongols

Ref. No.	Cells with Chromosome No. 45	46	47	48	Total
HIGH I.Q. MONGOLS					
85	-	6	69	-	75
135	-	1	28	1	30
FAMILIAL MONGOLS					
315	1	48	2	-	51
316	-	2	36	-	38
96	2	28	2	-	32
97	1	30	1	-	32
312	2	57	1	-	60
313	-	1	58	1	60
314	-	-	53	2	55
307	-	1	56	-	57
308	1	53	-	-	54
309	2	61	1	-	64
DOUBTFUL MONGOLS					
228	-	8	45	1	54
365	-	50	-	-	50
387	4	48	-	-	52
405	-	4	47	-	51
435	-	4	66	-	70

Discussion

Young mothers - The presence of a translocation in a parent prevents the chromosomes involved from pairing correctly during meiosis, and hence leads to the formation of a proportion of unbalanced gametes. Hamerton and Steinberg (1962) have reviewed the progeny produced by translocation-carrier parents of the 13-15/21 type in the published pedigrees. Female carriers are found to produce three classes of children, normal, translocation carriers and translocation mongols, in the ratio 1:1:1. The evidence in the case of the male carriers is not so unequivocal at present but there seems to be a tendency to produce an excess of carriers, and few mongols. There are not yet so many data available when the translocation is between two chromosomes of group 21-22. There are two possibilities: one, that two 21s may be involved, secondly that a 21 and a 22 are involved, and it is not possible to differentiate between them morphologically. 21/21 T carriers can only produce mongol progeny, while 21/22 T carriers are theoretically capable of producing normal, T carrier, and T mongol progeny.

Since such parents are usually young when their mongol children are born, it is obviously essential that young

parents of a mongol should have their chromosomes examined to see if either of them is a carrier. It was mainly for this reason that the present study of young mothers was undertaken; from this point of view there is no evidence as yet to suggest that it is necessary to study the child's chromosomes, when careful examination of both parents has shown only normal complements. For this reason the mongol child was not studied where there would have been difficulty in obtaining blood samples. Also, in one or two cases, the mongol child had died.

From the point of view of counselling parents, it is of importance to discover what percentage of young mothers are carriers of a translocation, and what percentage of translocations are inherited. In the present study of twenty such mothers only one translocation mongol was discovered, and both his parents had normal karyotypes. It would seem from this, and from the published results, that inherited translocations are relatively uncommon among mongols born to young mothers where there are no other affected relatives, most of the reported ones being spontaneous (Polani et al., 1960; Breg, Miller and Schmickel, 1961; Hamerton, 1962; Scherz, 1962; Dekaban, Bender and Economos, 1963; Haylock, 1963;

Sergovitch, Soltan and Carr, 1964). This is to be expected since, as Smith (1960) points out, young women are not really rare among the mothers of mongols although mongols are rare among the children of young mothers. Although the risk of any particular birth being affected rises with maternal age, the large number of children being born to younger women results in a proportion of mongols being born to them also.

If there is a mechanism at work here different from that in older mothers, it would not appear to be translocation as it is seen in familial cases. It is possible that some of the parents involved are mosaics of normal and translocated cell-lines, or of normal and trisomic cell-lines as the present patient No. 366, and that part or all of one or both gonads is composed of the abnormal cells. In the present study evidence was found of one such parent, and others have been reported in the literature, e.g. Verresen, van den Berghe, and Creemers, 1964; Weinstein and Warkany, 1963; Blank et al., 1962; Smith et al, 1962. Studies confined to peripheral blood always run some risk of missing a second stem-line which may be absent from the blood, and which in the present case was present in a very small proportion of the cells studied.

It is mainly among families with more than one affected individual that translocation chromosomes are found, and there are now many of these reported in the literature. None of the families of this type investigated in the present study proved to have other than chromosomally normal individuals, and the mongols were trisomic. However, in family C one of the mongols, and in family S both the mongols, were born when the mother was over thirty.

There is still a difference of opinion as to whether a mother with one mongol child has an increased risk of having a second one. Hamerton et al. (1961 a), in the series examined in conjunction with Carter, conclude, after removing the families in which translocation is involved, that some risk still remains. Penrose (1961) holds that this is difficult to prove, and that 'the risk of a second affected child is little more than double that expected in the population, taking maternal age into consideration'.

Mosaicism - Recently there have been a number of reports of mosaicism in mongolism; varying proportions of normal and trisomic, and in some cases more complex cell-lines, have been involved. The individuals concerned have varied from typically mongol, through genuinely doubtful cases where

mongol features but average intelligence were combined, or where some of the typical mongol traits were missing, to a case where a diagnosis of mongolism from the clinical features was remote (Richards and Stewart, 1962; Clarke, Edwards and Smallpiece, 1961; Fitzgerald and Lycette, 1961; Hayashi, Hsu and Chao, 1962). There are also the cases of mosaic mothers of mongols (see p. 78); those reported by Verresen, van den Berghe and Creemers, and by Weinstein and Warkany would not have been suspected on clinical examination to have a second cell-line. The phenotype will vary in each individual, depending on the proportion, distribution and constitution of the various cell-lines involved. Examination of atypical mongols is therefore of interest since, although the proportion of cells in various tissues is not necessarily that revealed by tissue culture methods, the different cell-lines present can be identified.

However, all the doubtful cases examined here proved to have unmixed cell-lines as far as can be determined from peripheral blood; the two cases with higher I.Q.s proved to have typical mongol chromosomes.

SECTION V

ABNORMALITIES OF THE SEX CHROMOSOMES

Chromosome studies have been of great value in the investigation of problems of abnormal sex development. Many unexpected results have been obtained, and new sex chromosome complements are still being uncovered in atypical individuals, which may help to elucidate the mechanisms involved in sex determination. Chromosome analysis can also prove of importance to the clinician in this field in particular; suspected errors of sex development can now be confirmed in infancy where previously this would not have been possible until puberty.

It has already been mentioned that although the human Y chromosome can be identified with confidence in a good preparation, the X is one of the most difficult chromosomes to identify directly. Autoradiographic studies are becoming increasingly important here, but information about the number of X chromosomes present can be indirectly obtained by the quicker method of studying the sex chromatin of the resting nucleus.

Sex Chromatin

This is the name given to the distinctive mass of chromatin found in the resting cells of the female in some mammalian species, and absent in the male (see Fig. 13). This sexual dimorphism in intermitotic nuclei was first observed by Barr and Bertram (1949) while studying some aspects of the behaviour of nerve cells in the cat; their preliminary observations indicated that a similar difference existed in the sympathetic ganglia of human males and females. These initial findings were extended to other tissues of the cat (Graham and Barr, 1952), to human tissues (Moore and Barr, 1954), and to many other mammals (Moore and Barr, 1953). Finally it was shown that it was possible to tell the sex of a human individual from the presence or absence of this body in the nuclei of cells of a skin biopsy (Moore, Graham and Barr, 1953). These sex chromatin bodies are sometimes referred to as Barr bodies.

The skin biopsy was the first material to be used routinely in nuclear sexing in humans (Hunter, Lennox and Pearson, 1954); it was followed by the simpler smear techniques (Moore and Barr, 1955). In humans the sex chromatin usually takes the form of a plano-convex mass,

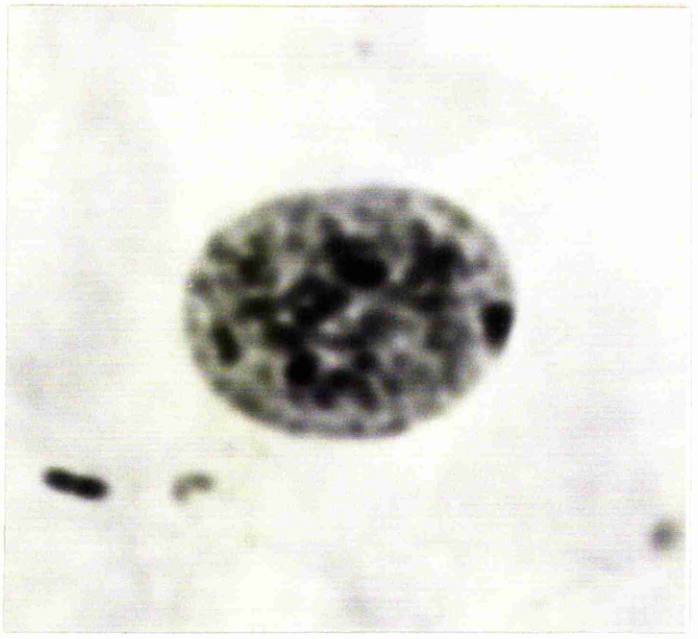
about 1 μ in diameter, lying against the nuclear membrane. It is distinctly larger than any of the other chromatin masses in the nucleus; in preparations of good quality from a normal female, it is usually visible in over 50% of the nuclei.

Sex chromatin is thought to be related to the chromosomes, since it is composed of DNA, and to the sex chromosomes in particular, since they alone are distinctly different in the two sexes. It is thought not to be a secondary sexual characteristic, since it has been shown to be quite independent of age and hormone status (Moore, Graham and Barr, 1957).

Originally the sex chromatin was thought to represent the paired heterochromatic regions of the two X chromosomes (Barr and Bertram, 1949), an interpretation which was supported by the fact that the body sometimes appears to be double; on the other hand it inferred somatic pairing of the chromosomes for which there is little evidence in mammals, although it is known to occur elsewhere (Boss, 1955). Another difficulty was the absence of a corresponding body of half the size in males. The discovery that sex chromatin negative Turner's syndrome and sex chromatin positive Klinefelter's

syndrome had sex chromosome complements of XO (Ford et al., 1959 a) and XXY (Jacobs and Strong, 1959) respectively was not incompatible with this interpretation, but the existence of an XXX female (Jacobs et al., 1959) with two sex chromatin masses was, since according to this theory four X chromosomes would be required to produce these two masses.

It now seems more likely that the sex chromatin represents one heterochromatic X chromosome. Studies of Ohno, Kaplan and Kinosita (1959) have shown that in the female rat one X chromosome is heterochromatic, and this observation has been extended to human cells (Ohno and Makino, 1961; Gilbert et al., 1962). The most likely explanation at the moment is that put forward in the Lyon hypothesis (Lyon, 1962), that early in embryogenesis one X chromosome becomes genetically inactive, and forms the sex chromatin body. In any cell of an individual this X chromosome can be either that of paternal origin or that of maternal origin, but once the differentiation has occurred in a cell the same X remains inactive in all descendants of that cell. This hypothesis has far-reaching genetic implications; for example it implies that the female has no more active genetic material than the male, and that a normal female is to be



(a)

(b)

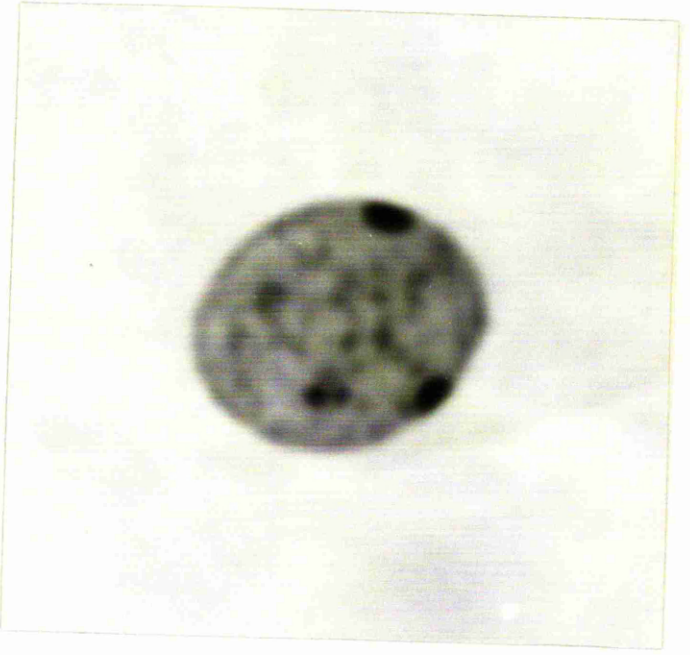


Figure 13. (a) Cell from buccal smear of a normal female, showing one sex chromatin body present.

(b) Cell from buccal smear of an XXX female, showing two sex chromatin bodies present.

regarded as a mosaic of the two X chromosomes, for which there is some supporting evidence (McKusick, 1964). It also accounts for the relatively small effect of the additional X chromosomes found in some disorders of sex development.

In any case the sex chromatin has been found to be an indispensable guide to the number of X chromosomes in the nucleus, there being one more X chromosome present than the number of sex chromatin bodies.

Patients with abnormalities of the sex chromosomes fall into three main groups:

1) those with one or more extra X but no Y (XXX females);

2) those with one or more extra X and at least one Y (Klinefelter's syndrome);

3) those with only one X (45 chromosomes in all) and those with one normal X and one X which is abnormal in some way (isochromosome, or deletion). These come under the heading of Turner's syndrome.

Chromosome Studies in the XXX Syndrome

In 1959 a new condition involving an abnormality of the sex chromosomes was described by Jacobs (Jacobs et al., 1959).

The patient, a female, had an unusually high proportion of nuclei with sex chromatin in her buccal smears, and some of the nuclei had two sex chromatin bodies. 47 chromosomes were found to be present; the extra element was in the 6-12 group and in view of the sex chromatin findings was interpreted as an X. This indicated the existence, so far only suspected on theoretical grounds, of a female counterpart to chromatin positive males (Klinefelter's syndrome).

It was obviously important that more individuals with this karyotype should be discovered if possible, to see if any definite clinical syndrome associated with the extra X chromosome could be established. It was also of interest to explore the frequency of this hitherto unknown condition. Since the XXY Klinefelter's syndrome has been shown to be more frequent among the mentally subnormal (Ferguson-Smith, 1958), it seemed possible that this might also apply to XXX females, and so a nuclear sexing survey was carried out on all the female patients of Lennox Castle M.D. Hospital, by Dr. Jean Fraser of this department. Buccal smears were taken from 595 patients; four of these were found to have a percentage of cells with two sex chromatin bodies (Fraser et al., 1960). A similar survey of girls in schools for the

mentally handicapped in Glasgow discovered two such individuals among 711 examined.

Chromosome studies were performed by the present author on five of these six individuals, in one case on sternal marrow material, and in the other cases on peripheral blood. In the sixth case the family proved uncooperative and no studies were possible.

In each case the modal number was 47; the extra chromosome was in the group 6-12, and was interpreted as an X with the help of the sex chromatin findings (see Table XV for chromosome counts, Fig. 14 for chromosomes). Chromosome analysis was also performed on peripheral blood material from the son of one of these women. His buccal smears were chromatin negative, and his chromosomes were apparently those of a normal male.

TABLE XV

Chromosome Counts in XXX Females

Ref. No.	Sex Chromatin	Chromosomes 45	46	47	48	Material
34	2 bodies	-	1	11	1	Peripheral blood
40	"	-	4	31	1	" "
44	"	-	6	28	3	" "
45	"	-	3	13	1	Bone marrow
111	"	-	4	35	-	Peripheral blood
43 (son of XXX)	negative	3	16	1	-	Peripheral blood

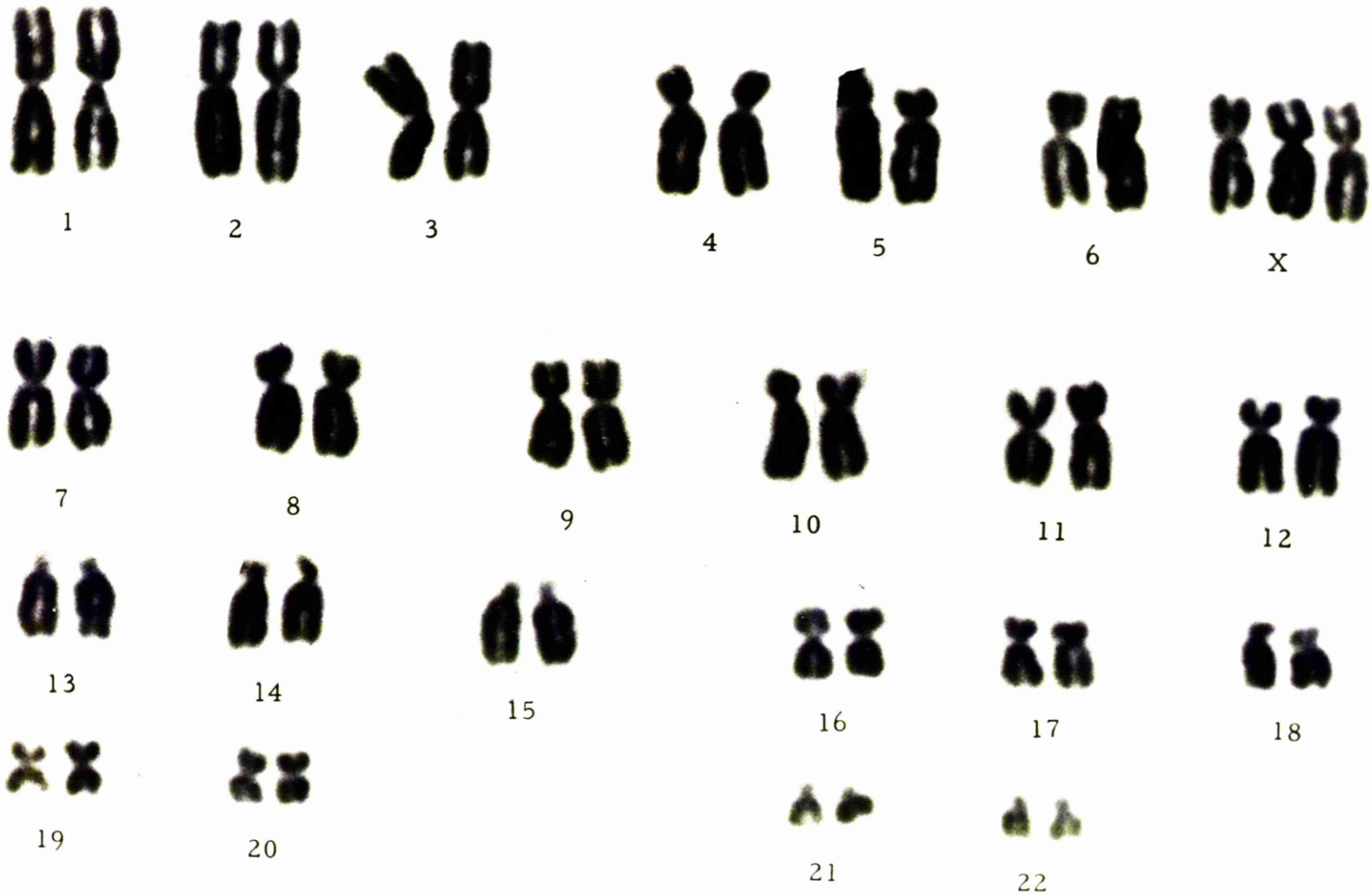


Figure 14. Karyotype of a cell from an XXX female.

Frequency

The frequency of XXX females among the patients at Lennox Castle was found to be 0·67%. Similar surveys have been carried out by Maclean et al. (1962) and by Johnston et al. (1961), and they found respectively 8/1907 (0·42%) and 3/827 (0·36%) women with two sex chromatin bodies; later surveys by Ridler, Shapiro and McKibben (1963) and by Day, Larson and Wright (1964) found 2/735 and 3/1088 XXX females among mentally retarded populations. This gives a total of 20/5152 or 3·88/1000.

In a nuclear sexing survey among newborn babies, Maclean, Harnden and Court Brown (1961) found four XXXs among 3000 female children; in a similar survey by Bergeman (1961) of 1383 females, none was found to have two Barr bodies. When these two sets of figures are combined they give a frequency among the newborn of 0·08%. It is thus obvious that, as is the case in Klinefelter's syndrome, the frequency of the XXX syndrome is higher among mentally defective populations.

Maclean et al. (1962), in summarising results obtained by various authors in surveys of mental institutions, concluded that the frequency of Klinefelter's syndrome with a single Barr body was 8·1/1000 (0·81%). His figure for

chromatin positive boys at birth (Maclean, Harnden and Court Brown, 1961) is 2·65/1000 (0·265%). Both figures are higher than in the female counterpart.

Clinical Features of XXX Syndrome

So far no syndrome has emerged by which individuals with sex chromosome constitution XXX can be recognised clinically. The original patient described by Jacobs was of normal intelligence; she had secondary amenorrhoea, underdeveloped breasts, her external genitalia were infantile and at laparotomy her ovaries appeared post-menopausal. However, these features are not common to other XXX females, of whom about 50 are now known. (The main features are summarised in Appendix B.)

Mental Status - From the results of the nuclear sexing surveys previously discussed it appears that the frequency of XXX females is higher in mentally defective populations than in the general population. Only four such patients have been reported with normal intelligence; the remainder have been described as subnormal in various degrees. However, they have almost all been discovered in the course of surveys of institutions for the mentally subnormal, so these figures

cannot be regarded as giving a true picture of the percentage of mental defectives among XXX individuals. At present there is no information concerning the frequency of XXX individuals in the general population, except for the survey of newborn babies by Maclean, and it will be some time before there is any information about the mental status of the babies there discovered to be XXX.

Of the four patients at Lennox Castle, cases 2 and 4 did not differ in any striking way from the general run of 'non-specific' mental defectives. Case 4 showed marked intellectual deterioration to a degree greater than would be expected from her age alone, but this again was of an unspecific nature and was not considered to be significant. She was admitted to the institution in 1916 at age 30; case 2 was admitted in 1943 when she was aged 22 years. Case 3, who was admitted in 1937 at age 38 years, has some evidence of schizophrenia in addition to the mental defect, while in case 1, admitted in 1949 at age 19 years, the original diagnosis of mental defect was changed to one of manic depressive psychosis.

In a psychiatric investigation of 22 Scottish XXX females in mental institutions, which presumably includes the

above four patients, and many of those listed by Court Brown et al. (1964), Kidd, Knox and Mantle (1963) found that only 11 were mentally subnormal, the other 11 suffering from psychotic illness. The diagnoses of the latter were typical of the general range found in the hospital in which they were located, but with a greater degree of withdrawal and more severe impairment of inter-personal relationships. Among the sub-normal patients the percentage with psychoses was unexpectedly high.

Day, Larson and Wright (1964) have pointed out that among the XXX females who have been described, there are several whose mental condition could be accounted for by factors other than the extra X. For example, one of the cases he himself describes is cretinoid, while another has a family history of delinquency and mental defect. Family history of mental retardation is shown by one case of Johnston et al. (1961), while another case of his has family history of schizophrenia, and in his third case there is a question of toxoplasmosis. Such cases appear to be a minority. None of the four cases reported by the present author had any relevant family history or other apparent cause. It seems likely that the extra X either causes mental retardation in the majority of cases, or makes mental illness more likely.

Genital Tract - Four of the known XXXs have had secondary amenorrhoea and another 4-5 have had irregular periods. The remainder have menstruated normally, and several are of proved fertility. The menstrual history of the oldest XXX patient from Lennox Castle is unknown, but in the other three it is more or less normal, and one has given birth to a son. An affected female who had 9 pregnancies is reported by Barr and Carr (1960); similarly, an XXX female described by Stewart and Sanderson (1960) has had 5 children, and one described by Breg, Cornwell and Miller (1962) has had 4 children; and several other offspring born to XXX mothers have been reported - about 30 in all. It is remarkable that in all cases where investigation has been carried out these children have had normal chromosome complements, whereas 50% with an extra X would have been expected theoretically (as in the case of children born to mongol mothers [Hamerton, 1962]).

All four patients at Lennox Castle had unremarkable external genitalia, and this applies to all the reported cases with the exception of Jacobs' first case, where they were infantile. Thus the presence of the extra X chromosome produces little consistent effect on the genital tract in the female - a direct contrast with XXY males, who are sterile.

Other features - All four Lennox Castle patients were epileptics, but this is probably coincidence, as is the finding that all are blood group A.

The height of the reported cases ranges from 4'9" to 5'9¼", but the majority are average and there is no stunting of growth such as is observed in Turner's syndrome. Similarly the relative proportions of the body are normal; there is no effect comparable to that shown in XXY Klinefelter's syndrome (Johnston et al., 1961).

Thus, no consistent clinical features emerge either from the reported cases or from studies of our own patients, which also included analysis of cerebro-spinal fluid and serum, X-ray examination, electroencephalogram, electrocardiogram, blood grouping and family history investigation.

Additional Xs

Carr, Barr and Plunkett (1961) described two mentally defective females of sex chromosome constitution XXXX (48 chromosomes). Both had apparently normal menstrual history; the first had reproductive system normal for a girl in early adolescence (14 years), and the second (aged 33 years) had normal external genitalia and vagina, though no ovaries could be felt. Both were of average height.

An infant with 49 chromosomes and sex chromosome constitution XXXXX has been reported by Kesaree and Woolley (1963). Developmental progress is severely retarded, and there are associated congenital abnormalities. This effect is comparable to XXXY and XXXXY cases - see Klinefelter's syndrome.

Klinefelter's Syndrome

This is a not uncommon condition which has been estimated to account for about 5% of all subfertility in males , the chromatin positive variety accounting for 3% (Ferguson-Smith et al., 1957). The main feature is the small size of the testes; histologically these show an increased number of Leydig cells, and underdevelopment of the seminiferous tubules, with germ cells much reduced or absent. The patients are sterile. There may also be gynaecomastia, increased urinary output of gonadotrophins, and reduced output of 17-ketosteroids.

Bunge and Bradbury (1956) reported that some of these cases were chromatin positive, and Ferguson-Smith et al. (1957) pointed out that it was possible to differentiate between the chromatin positive and the chromatin negative cases by the histopathology of the testes, that of the chromatin positive

type - about 40% - being more grossly abnormal. Nuclear sexing surveys of the newborn have shown the following incidence of males with one sex chromatin body: 5/1911 (Moore, 1959); 4/1890 (Bergeman, 1961); 9/3000 (Maclean, Harnden and Court Brown, 1961). This gives a frequency of 18/6801, 0·26%.

A proportion of these patients are mentally defective; chromatin positive Klinefelter's syndrome has been found in 1·2% of all male inmates of a hospital for the mentally deficient (Ferguson-Smith, 1958), and in the same percentage of mentally handicapped schoolboys (Ferguson-Smith, 1959).

Chromosome Complement in Klinefelter's Syndrome

Chromatin negative cases. Seven cases reported by the Edinburgh group (Court Brown, Jacobs and Doll, 1960) were chromosomally normal males, as were the three patients studied here (see Table XVI for chromosome counts). These chromatin negative cases probably represent a variety of lesions damaging the testis in the same sort of way, some a congenital defect of germ cells not due to chromosomal defect, and some due to damage of the testis after birth, e.g. by mumps.

Chromatin positive cases. Jacobs and Strong (1959) were the first to report that the chromosome constitution was

XXY and this has since been fully confirmed by other workers. Two other karyotypes have been reported:

XXYY

Muldal and Ockey (1960) report an individual of this karyotype who was apparently clinically indistinguishable from the XXY patients. Two similar cases were reported by Maclean et al. (1962), and one by Carr, Barr and Plunkett (1961). This patient was also diagnosed as chromatin positive Klinefelter's syndrome, but did show unusually severe regressive changes in the testis.

Mosaics

Two typical chromatin positive cases turned out to be XX/XXY (Ford et al., 1959 b; Crooke and Hayward, 1960), and one to be XY/XXY (Tough et al., 1961). Also two cases of Nowakowski et al. (1960) were 47 XXY, while a proportion of cells were 46, XX or XY, and a few were 45 XO. More individuals displaying mosaicism have since been described (Court Brown et al., 1964).

As these cases were clinically indistinguishable from XXY cases the author, while not attempting a large survey, has examined the chromosomes of fifteen patients with Kline-

felter's syndrome, in the course of which three individuals of interesting karyotype were discovered (see Table XVI of chromosome counts).

TABLE XVI

Chromosome Counts in 15 Patients with Klinefelter's Syndrome

Ref. No.	Nuclear Sex	Cells with Chromosome No. 45	46	47	48	49	Karyotype
276	negative	2	33	-	-	-	XY
279	"	7	36	-	-	-	XY
401	"	6	38	-	-	-	XY
151	positive	-	-	24	1	-	XXY
157	"	-	4	35	-	-	XXY
179	"	2	11	43	-	-	XXY not mosaic
264	"	-	3	36	2	-	XXY
274	"	-	3	36	-	-	XXY
280	"	2	-	38	-	-	XXY
282	"	-	3	51	-	-	XXY
283	"	-	2	33	1	-	XXY
403	"	-	3	47	-	-	XXY
273	positive	5	67	2	-	-	XX
281	2 Barr bodies	-	4	14	81	1	XXXY/XXY
113	2 " " and some 3 " "	-	-	2	2	41	XXXXY

All the chromatin positive cases, with the exception of No. 273, had unmixed XXY karyotypes (see Fig. 15). In No. 179 all the 11 cells with 46 chromosomes were fully analysed, but there was no evidence of mosaicism, and it seems likely that these were broken cells, although this is a much larger proportion than is normally observed.

Patient No. 273

This patient was diagnosed as chromatin positive Klinefelter's syndrome with gynaecomastia. He is described as a feminine-looking boy whose voice broke at 18 years and who first shaved at 20 years. He is 5'6" tall with arm span 5'5½". Both testes were about the size of a pea. Histologically, the majority of the seminiferous tubules were small and completely hyalinised; the remainder were of approximately normal size but lined solely with Sertoli cells. There was no evidence of spermatogenesis. Chromosome counts were performed on peripheral blood, and the karyotype proved to be XX. Chromosome counts performed by Dr. M.A. Ferguson-Smith on cells from a skin culture also showed an XX chromosome pattern.

Therkelsen (1964) has published a report of a comparable patient, again with XX chromosome constitution, and a similar patient has been described by de la Chapelle *et al.* (1964).

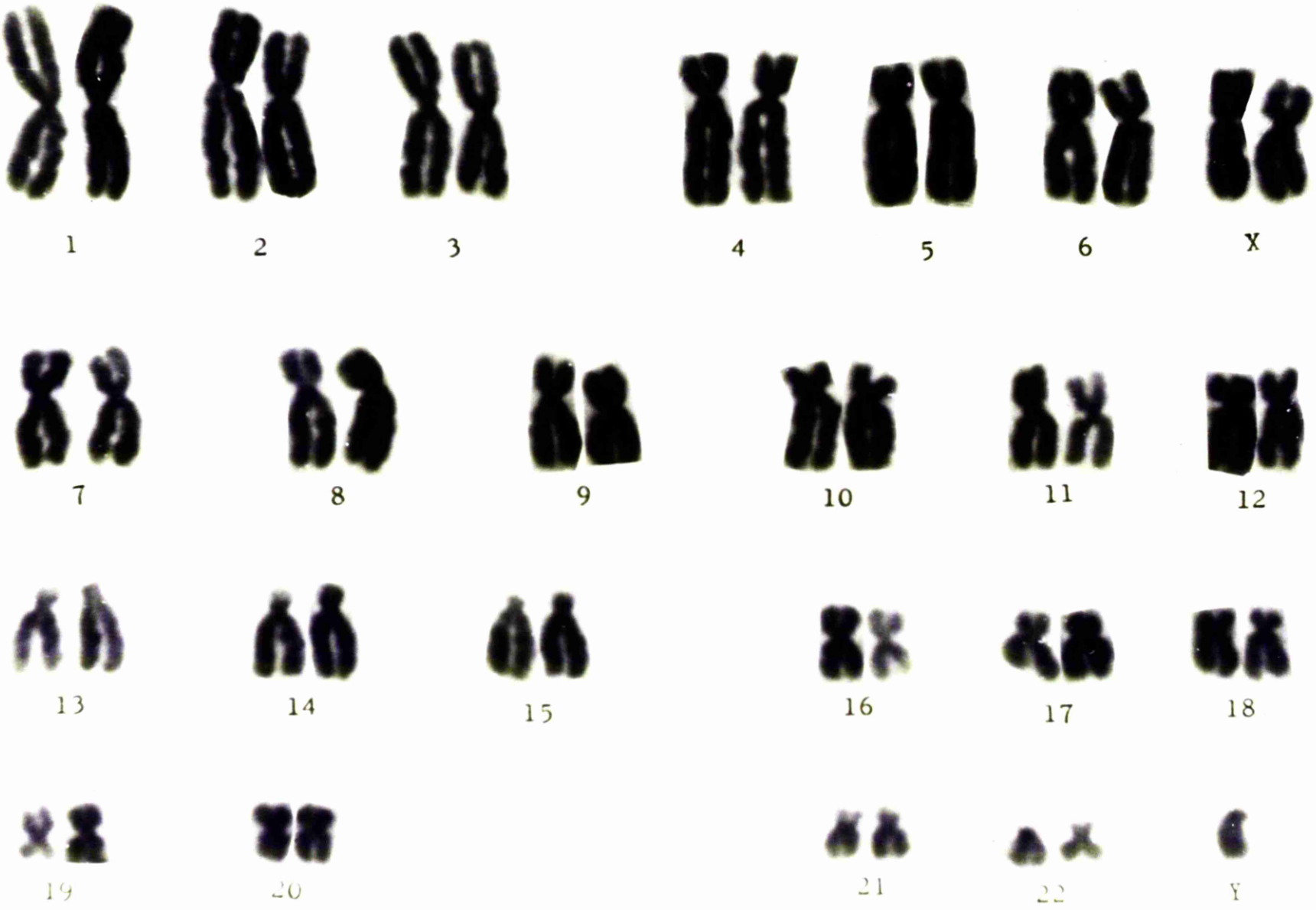


Figure 15. Karyotype of a cell from a patient with Klinefelter's syndrome, showing XXY sex chromosome constitution.

Patient No. 281

The chromosomes of this patient were studied because of the presence in his buccal smears of a proportion of cells with two sex chromatin bodies. The majority of cells counted had 48 chromosomes and were interpreted as having XXXY sex chromosome complement (see Fig. 16). The presence, among the 14 cells with 47 chromosomes, of 10 cells whose karyotype was apparently XXY makes it likely that this patient was an XXY/XXXY mosaic. Unfortunately his family proved uncooperative and no further investigation was possible.

Two cases of XXXY Klinefelter's syndrome are described by Ferguson-Smith, Johnston and Handmaker (1960) and a further three by Maclean et al. (1962). The sex differentiation is the same as in XXY Klinefelter's syndrome; the mental defect is apparently more severe, and one case shows associated congenital malformations.

Patient No. 113

Again, chromosome investigation of this patient was undertaken because of the presence in his buccal smears of nuclei with two, and occasionally three, sex chromatin bodies. 49 chromosomes were found to be present, and the sex chromatin complement interpreted as XXXXY (see Fig. 17). In this case the cell-line was unmixed. A report on this case has been published (Fraser et al., 1961).

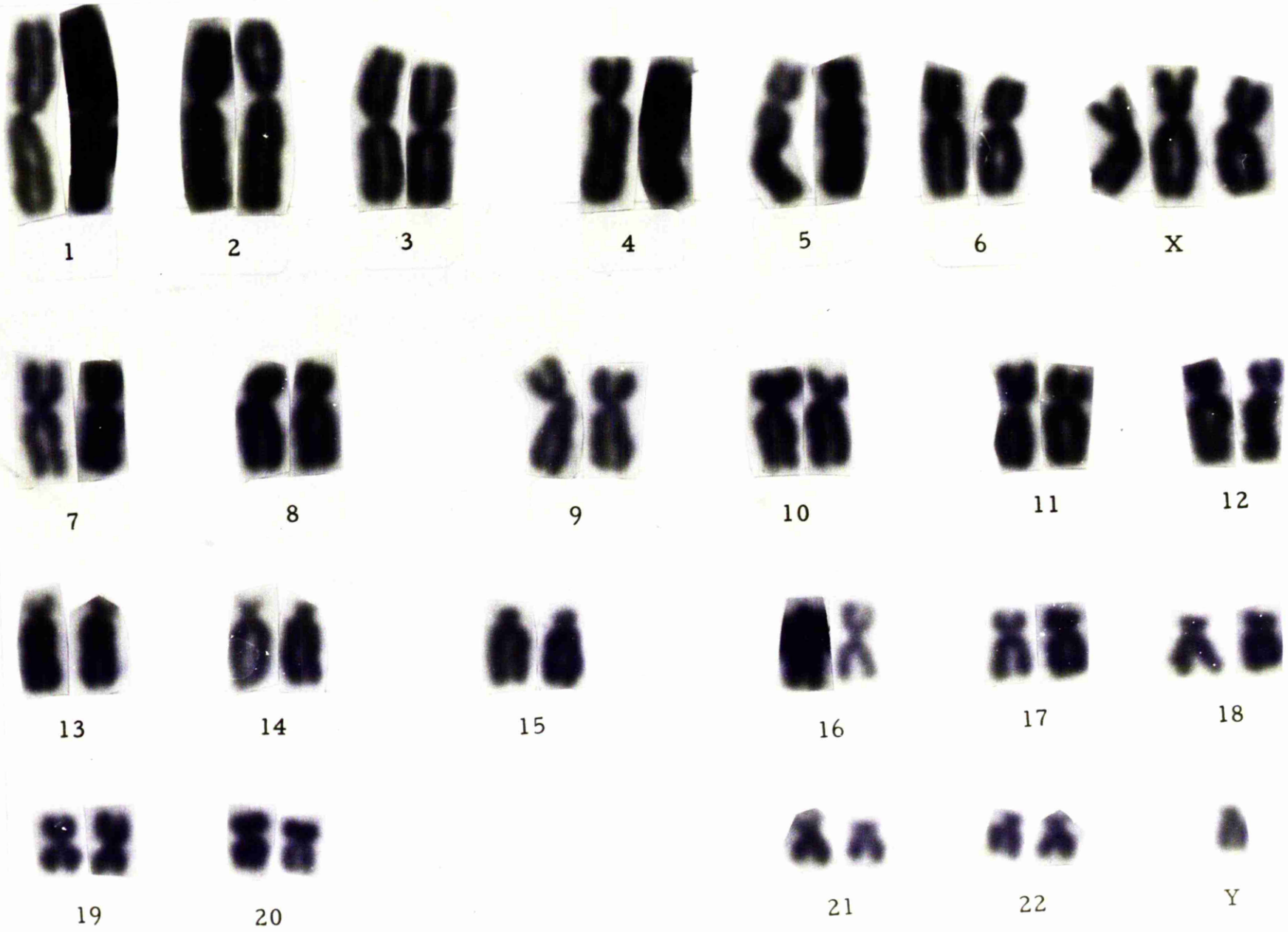


Figure 16. Karyotype of a cell from patient No. 281, showing XXXY sex chromosome constitution.

Other individuals of this karyotype have since been reported; some of these are summarised in Appendix C. The abnormalities associated with this condition are more severe than is the case with XXY and XXXY types.

Mental Defect - The present patient, a boy of $8\frac{1}{2}$ years, had a mental age of 2 years 8 months on the Merril-Palmer scale, and was considered to be ineducable. Barr's second case, a baby of 7 months, had a mental age of 4-5 months; Fraccaro's first case was probably retarded, and the remaining cases have had low I.Q.s. Thus fairly severe mental defect is evident in all cases.

Genitalia - In the present case the penis was normal, but in the majority of others it is small. In all except Barr's first case the scrotum is hypoplastic. Both these features contrast with XXY and XXXY.

Testes - Again, these are more abnormal than in the other variants. In only one case (Atkins et al., 1963) are the testes completely descended; they are always small, and in two cases not palpable at all. In four cases testicular biopsies have been performed. Three of these - including the present case - show scanty tubules with no evidence of germ cells, while the fourth, that of the 7 month old baby

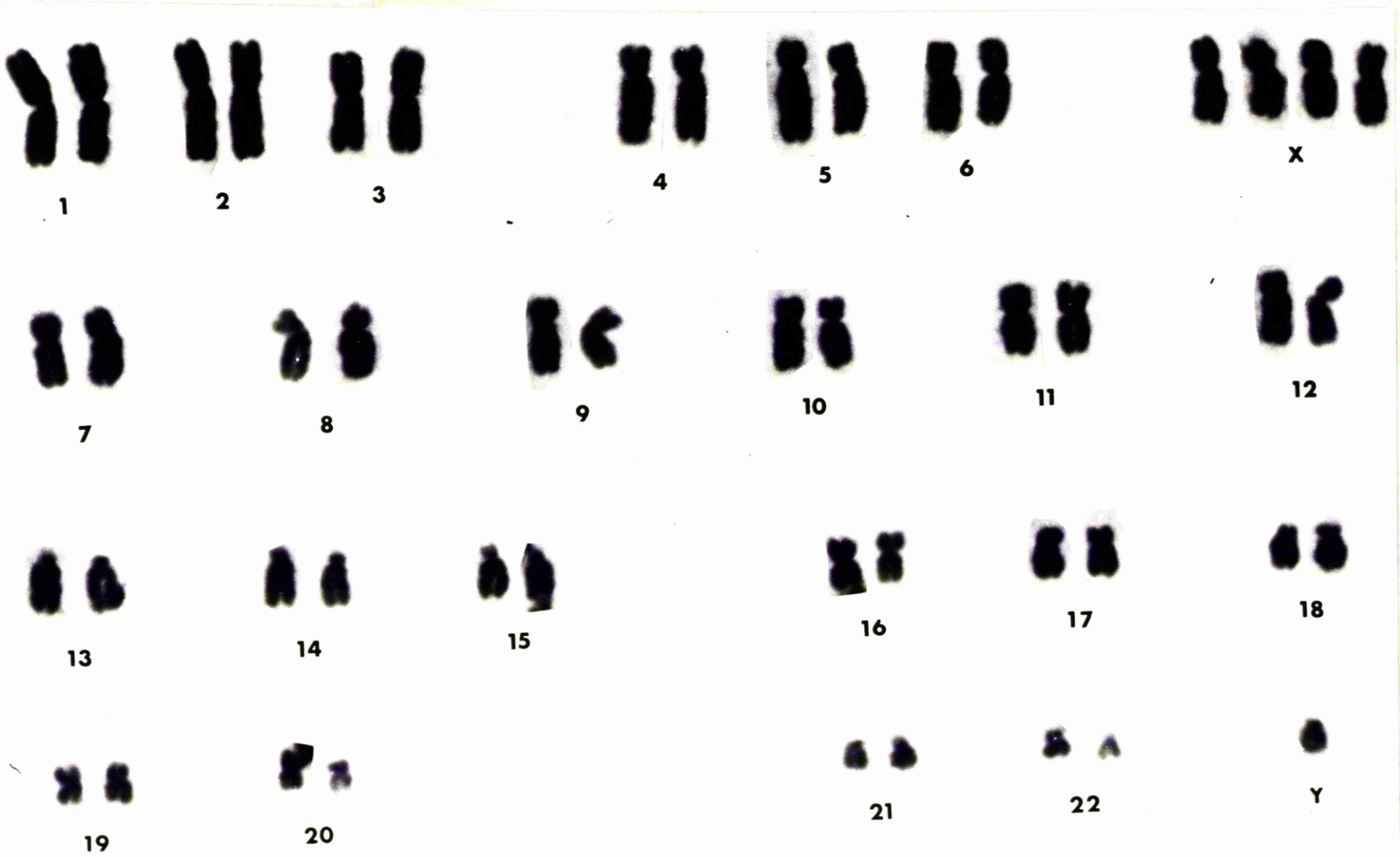


Figure 17. Karyotype of a mitosis from patient No. 113, showing XXXXY sex chromosome constitution.

reported by Barr, was approximately normal for his age but had sparse and degenerating spermatogonia.

Skeletal Deformities - These are shown by all patients. In the present case the sella turcica is enlarged and deep; there is bilateral coxa valga - also shown by both Miller's and Anders' cases. The proximal parts of both ulnae are expanded, and the radius on both sides is elongated proximally. This is also a feature of Miller's case. Radio-ulnar synostosis is present on the left side - also seen in Fraccaro's second case, Anders' and Joseph's cases, and Barr's second case, which in addition has short bowed shafts in both radius and ulna. Our patient has abnormal ossification centres in the hands, and the terminal phalanx in each little finger is incurved, as in Joseph's case and Fraccaro's second case. His first had centres of ossification missing in multangulum minus and capitulum radii, while that of Anders has multiple pseudoepiphyses of hands and feet. Also two cases had flat feet, and two had abnormalities of the spine. Retardation of bone age has also been observed in several cases. Thus skeletal deformities are associated here to a greater extent than in the other variants.

Effect of Extra Xs - The presence of an additional X chromosome leads, in the female, to an increased incidence of mental defect, and little other effect; in the male, to sterility and again increased mental defect. Additional extra Xs (XXXY, XXXXY) result in increasingly severe mental defect, and in associated congenital abnormalities.

Turner's Syndrome

This is a disorder in women with many variations and complexities, but the most characteristic feature is non-development of the ovaries; in the position these usually occupy are found streaks of ovarian stroma which contain no follicles. The rest of the germinal tract is that of a normal female, but it always remains infantile. There is no development of secondary sex characteristics; there is increased urinary excretion of gonadotrophins, and the urinary output of 17-ketosteroids is reduced. Such patients are generally amenorrhoeic, although some cases have been known to menstruate irregularly for some time (Hoffenberg, Jackson and Muller, 1957) and one exceptional patient has given birth to a son (Bahner et al., 1960).

Patients with this condition are usually of short stature and may show a wide assortment of congenital abnormalities including webbing of the neck, coarctation of aorta, shield chest, abnormal development of the fingers and toes, and peripheral lymphedema in early life, when it may be of value in diagnosis in the newborn. There is a wide range of variation between individuals in these associated abnormalities.

Sex Chromatin and Chromosome Studies

In 1954 it was shown by Polani, Hunter and Lennox that a proportion of patients with Turner's syndrome had no sex chromatin bodies in the cells of their skin. Indirect evidence of colour-blindness studies suggested that only one X chromosome was present (Polani, Lessof and Bishop, 1956) but blood group studies (Platt and Stratton, 1956) ruled out haploidy of the whole chromosome set. Then it was suggested (Danon and Sachs, 1957) that the sex chromosome constitution was XO and this was confirmed when Ford et al. (1959 a) reported finding 45 chromosomes in a patient with Turner's syndrome, with the probable sex chromosome complement of XO. Many of the patients described as having Turner's syndrome,

and discovered to be chromatin positive, have had most interesting sex chromosome constitutions. Occasionally two apparently normal chromosomes were present; several have one normal X and one which is structurally abnormal, and others have been mosaics, often with an XO cell-line and a second cell-line with a structurally abnormal X. All these variants are fully discussed in a recent paper by Ferguson-Smith et al. (1964).

This is however a very rare condition, estimated to occur with a frequency of 0·37 per 1000, and it is difficult to accumulate a series of patients. The present studies have been confined to chromosome examination to help in clinical diagnosis (see Table XVII for chromosome counts).

TABLE XVII

Chromosome Counts in Patients with ? Turner's Syndrome

Ref. No.	Cells with Chromosome No. 44	45	46	47
67	-	3	21	-
105	-	3	37	1
140	2	18	17	-
356	2	48	-	-
409	-	-	51	1
423	-	3	47	-
425	7	59	55	2
427	1	48	1	-
437	-	1	50	-

Patient No. 425

A 19 year old girl with growth failure. There is no history of maternal illness during pregnancy. Birth was normal and dietary history was adequate at all periods of her life.

She is of average intelligence, of height 4'5½" and weight 5 st., with normal adult proportion despite her small size. She has a peculiar round face, with narrow spacing between eyes, and broad chest out of proportion to her height. Her limbs are short and she has broad hands and fingers; also waddling gait and inturning feet. She was treated at age 5 for congenital dislocation of hip and later for genu varum. Bone age 11 years 11 months.

No menarche has occurred; her breasts are pre-pubertal and underdeveloped; no axilla or pubic hair is present and there is no evidence of maturation of external genitalia. The initial diagnosis was of hypopituitary dwarfism.

Buccal smear showed only 5% chromatin positive nuclei. It was suspected that there was an abnormality of the sex chromatin body but the smears were not of very good quality. Chromosome examination showed the presence of two cell-lines; one 45 XO, the other had 46 chromosomes, and apparently three

members of pair number 3. This was interpreted as an iso-chromosome of the long arm of the X (see origins of abnormal chromosomes, and see Fig. 18).

Patient No. 140

A female of broad stunted growth who was diagnosed as incomplete Turner's syndrome. Height 4'6½", arm span 4'8½". No breast development, no axillary or pubic hair. The clitoris was enlarged, vagina was present, there was a very small cervix and uterus and no evidence of ovaries. Primary amenorrhoea. Gonadotrophins >40 <80 m.u./24 hours.

Cubitus valgus was present. The patient was pigeon-chested and had ventricular defect, but no webbing of the neck or fingers.

Buccal smears were chromatin negative. Of the 35 cells counted, 18 had 45 and 17 had 46 chromosomes. 5 cells from each cell-line were analysed to make sure the chromosomes were consistent; those with 45 chromosomes had only 15 in the 6-12 group, and were presumably XO, while those with 46 were XX.

Patient No. 356

A chromatin negative female aged c. 5 years. She had oedema of feet, legs and hands and was dwarfed. She also had

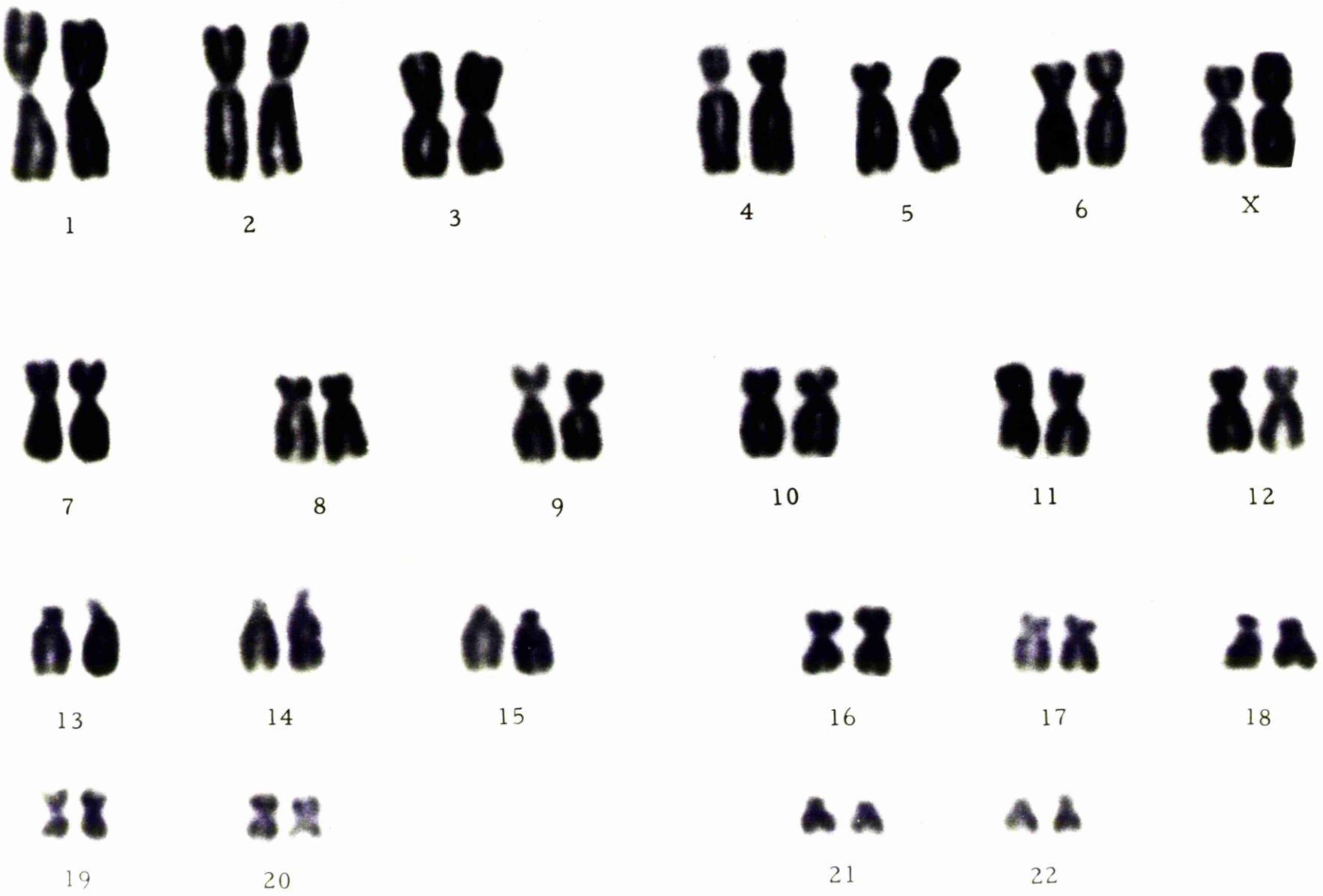


Figure 18. Karyotype of a cell from patient No. 425, showing the presence of an isochromosome for the long arm of the X.

a webbed neck and short fingers. On chromosome study she proved to have XO karyotype (see Fig. 19).

Patient No. 427

A chromatin negative girl aged 10 years, with an odd facies and low hair line. No finger clubbing and no neck webbing were present, but she had oedematous hands and feet. Examination showed 45 chromosomes to be present with sex chromosome constitution XO.

Patient No. 67

A baby of four months with webbing of the neck and lowered hairline. She had long fingers, large and prominent ears, and there was a haemangioma on the occiput. There was also a congenital abnormality of upper dorsal vertebrae.

Patient No. 105

A $3\frac{1}{2}$ year old girl with ventricular septal defect and webbed neck.

Patient No. 409

A chromatin positive female with primary amenorrhoea, and no vagina or uterus.

Patient No. 423

A 19 year old female with primary amenorrhoea and no vagina. The first buccal smear suggested that an abnormally

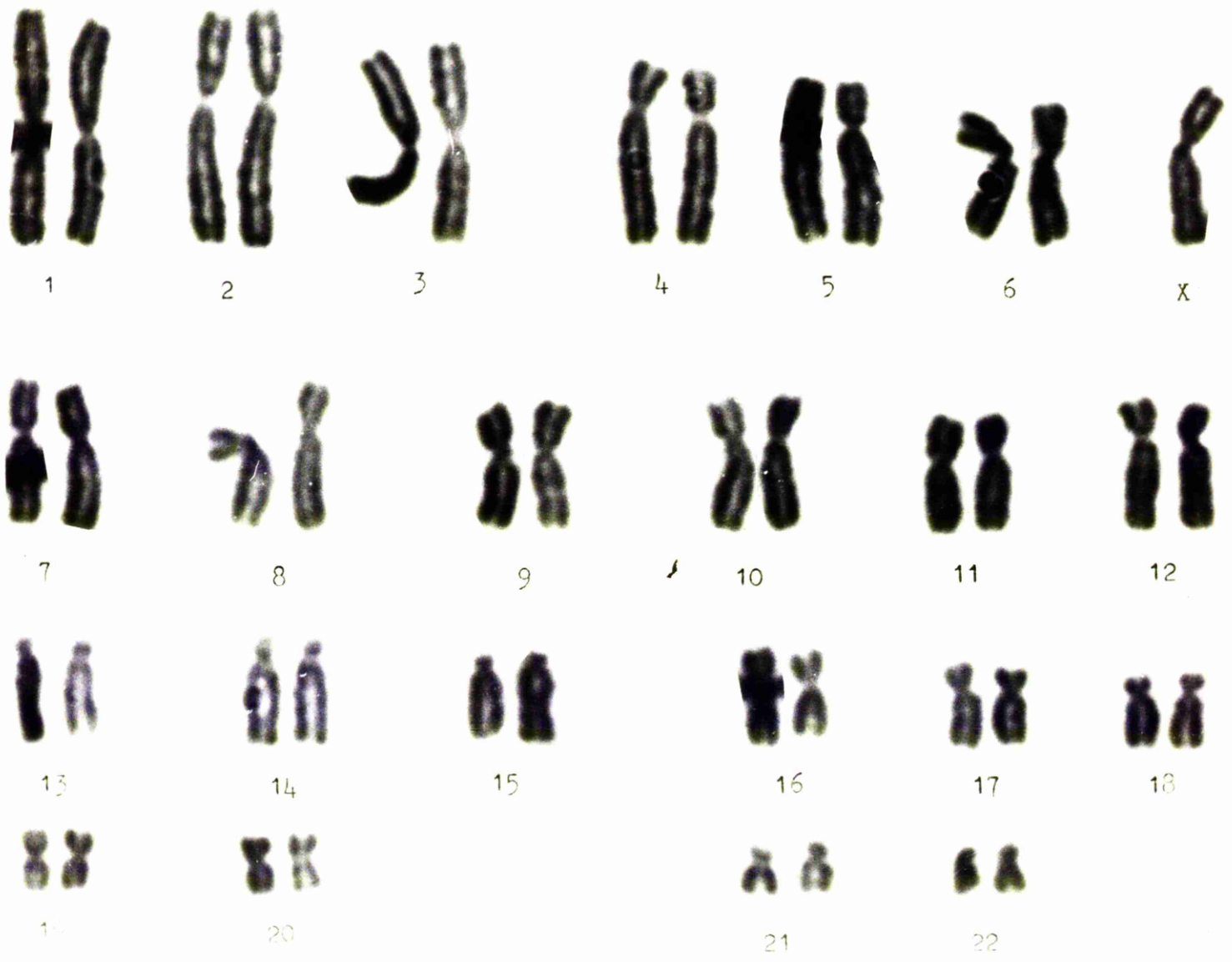


Figure 19. Karyotype of mitosis from a patient with XO Turner's syndrome.

low percentage of sex chromatin bodies might be present, but when repeated on a better smear this was found to have been incorrect.

Patient No. 437

A 6 year old girl with stunted growth. She had epicanthic folds, a large tongue and high palate, and webbing of the neck. She also had an increased carrying angle.

Patients Nos. 67 - 437

Chromosome studies in these six patients showed a full complement of 46 to be present, and no abnormality of either X chromosome could be recognised. Knowledge of the chromosome constitution thus provides additional information for diagnosis.

Origins of Abnormal Chromosomes

I. Numerical Abnormalities

Non-disjunction

When the two chromosomes of a bivalent in the first meiotic division fail to separate, or when they fail to pair at all, so that both members of the pair are included in the one daughter cell, this is known as primary non-disjunction and results in gametes with the abnormal chromosome numbers n - 1, n + 1. A similar error can occur involving the daughter chromosomes formed by longitudinal splitting of the centromere in mitosis or second meiosis.

Gametogenesis in an individual who has an abnormal number of chromosomes to start with, e.g. an XXX female or a trisomic mongol, must inevitably involve non-disjunction - this is described as secondary non-disjunction.

Also, it is possible for one daughter chromosome to lag behind when the others separate, thus failing to be included in either cell, and giving the products n - 1, n. Such a lagging chromosome could be included in the wrong daughter cell, giving n - 1, n + 1, an end product indistinguishable from non-disjunction.

Chromosomally abnormal individuals with one stem line

(1) Errors involving a single event, e.g. XO, XXX, XXY.

The most likely explanation is primary non-disjunction in gametogenesis in one of the parents.

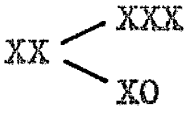
XO - product of an X bearing gamete from one parent and a gamete with no sex chromosome from the other.

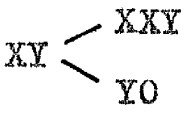
XXX - could result from either XX ovum produced by non-disjunction at either first or second meiosis, or from XX sperm produced by non-disjunction at second meiotic division.

XXY - XX ovum produced as above, or XY sperm from non-disjunction at first meiosis.

If there is some associated indirect evidence, such as the incidence of colour blindness where the X chromosome is involved (Lennox, 1961), it may be possible to deduce in which parent the error arose.

It is also possible to explain these karyotypes as products of mitotic non-disjunction at the first division of a normal zygote, where the other cell-line has failed to develop.





(inviable)

(2) Errors involving more than one event, e.g. XXXY, XXXXY.

(a) XXXY could be produced in a number of ways:

(i) primary non-disjunction in both parents - XX ovum + XY sperm;

(ii) non-disjunction in both meiotic divisions in either parent - X ovum, XXY sperm, or XXX ovum, Y sperm;

(iii) non-disjunction of one X at first division of an XXY zygote where the XY cell-line has failed to develop.

(b) XXXXY - again there are several possibilities:

(i) non-disjunction in both meiotic divisions in one parent, and in one meiotic division in the other - XXX ovum, XY sperm, or XX ovum, XXY sperm;

(ii) non-disjunction of both Xs in both meiotic divisions in mother - XXXX ovum, Y sperm;

(iii) non-disjunction of both X chromosomes in the first division of an XXY zygote, the YO product being inviable.

In view of the fertility of XXX females, secondary non-disjunction resulting in the production of an abnormal ovum must be considered in these cases. Unfortunately it was not possible to examine the parents of either our XXXY or XXXXY patients, but in other reported cases the parents have had normal chromosome constitutions.

(3) Mosaics

Mosaics of the type XO/XXX probably arise from mitotic non-disjunction at the first division of an XX zygote, and both cell-lines have developed.

Where there are more than two cell-lines, e.g. XO/XX/XXX, non-disjunction has probably occurred in the early divisions - but not the first - of the XX zygote.

Mosaics such as XX/XXX can either arise as above, if the XO line fails to develop, or may be due to one X lagging in the division of an XXX zygote, and failing to be included in either daughter cell.

The phenotype produced by a mosaic will depend on the distribution of the various cell-lines throughout the body. Thus one combination of cell-lines may give rise to different clinical symptoms in different individuals.

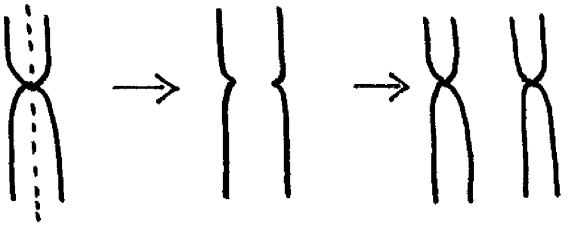
Maternal age effect in non-disjunction

The well known maternal age effect in mongolism (see section on mongolism) suggests that non-disjunction is increased in older mothers. A similar but less marked effect is shown by XXY and XXX syndromes (Polani, 1962), though not apparently in Turner's syndrome.

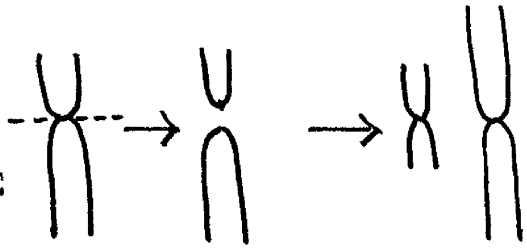
II. Structural Abnormalities

The only one of relevance here is the isochromosome of the long arm of X. An isochromosome is a completely metacentric chromosome. It arises by misdivision of the centromere, which splits at right angles to its usual direction of division.

NORMAL



ISOCHROMOSOME



One arm is thus represented twice, the other not at all.

SECTION VI

CHROMOSOME STUDIES ON PATIENTS RECEIVING ^{131}I THERAPY

Introduction

Roentgen discovered X-rays in 1895. The potentiality of X-ray photographs in surgical diagnosis was soon realised, but the fact that living tissues could be damaged by the passage of X-rays through them was not appreciated until later. The first deleterious effects to be noticed - changes in the skin, and epilation - themselves led to the development of X-ray therapy for superficial lesions; it was later realised that X-rays could also cause malignant disease. Similarly, naturally occurring radiations due to decay of radioactive material, first discovered by Becquerel in 1896, were found to produce skin burns and other biological effects.

The interaction of these radiations with living tissues is extremely complex, and is not well understood at present. Several stages are involved. Initially, the energy of the radiation is transferred to the tissue; this is brought about in one of two ways. The first of these is by causing an electron to be ejected from an atom, which thus becomes

positively charged, and is known as an ion - hence the name of ionising for this type of radiation. The ejected electron eventually becomes attached to another atom, forming a negative ion; in the meantime it may cause ionisations on its own. This reaction is biologically the more important method by which energy is absorbed by the tissue through which the ionising radiation is passing. The second method involves a smaller energy transfer; an electron in an atom is raised to a higher state of energy without actually being ejected. Such an atom is referred to as being 'excited'.

Since molecules are held together by electrical forces, these primary products of the passage of radiation are very unstable and reactive; they immediately undergo secondary reactions. Ionised molecules may react with other molecules, or may undergo internal reorganisation; excited molecules may dissociate spontaneously. The product of this very brief period of reorganisation is a mixture of stable molecules, some of which may be substances foreign to the cell, and chemically unstable fragments, such as free radicals. Very important among these products are the peroxides and free radicals produced by the ionization of water, since water comprises about 75% of the living cell.

There follows a period of chemical reactions, when the free radicals and other reactive elements react with each other and with other cell constituents to give the final chemical products. The molecules of which the cell is composed are thus liable to damage in two ways; first, directly by the radiation itself, and secondly as a result of the consequent chemical reactions. The metabolism of the cell is impaired by these changes, for example by the inactivation of an enzyme, and this leads to inactivation or possibly to death of the cell, or to inhibition of cell division.

Effect on the chromosomes

One effect of radiation on cells already in division is to bring about an alteration in the surface properties of the chromosomes, causing them to become 'sticky' (Lea, 1955). This means that chromosomes coming into contact at metaphase will adhere to one another, and when they begin to separate as cell division continues, bridges will be formed between the two daughter groups of chromosomes which must break before the separate daughter nuclei can be formed. In severe cases the chromosomes may become clumped together at metaphase and

the division proceeds no further. This effect is thought to be brought about by the depolymerization of the DNA.

A second general effect, this time exhibited by cells nearing the end of interphase at the time of irradiation, is a delay in entering mitosis. When the cell does divide, which may be several hours later, there is no sign of the 'sticky' effect.

Probably the most far reaching effect of ionising radiations upon the chromosomes is the production of changes in the genes themselves. Such mutations (which are generally harmful) are passed on to the daughter cells at division, and should they occur in a germ cell, will be passed on to the offspring produced. This effect of radiation, first discovered by Muller in 1928, has been extensively studied in Drosophila.

Radiation can also bring about structural changes in the chromosomes. The passage of an ionising particle through or near the chromosome thread may break it; when this happens the broken ends retain for a while the ability to join together again, and this they frequently do, reforming the original chromosome. If the broken ends do not join again the result is a deleted chromosome and an acentric fragment. These

fragments lack a centromere, and so lack the means of separating to opposite poles at mitosis. They lag behind the other chromosomes and often fail to be incorporated in the daughter nuclei. Thus they usually become eliminated in the course of a few mitoses.

The broken ends of a chromosome may join up in a different fashion, so that it contains the same material as before but the linear arrangement is altered. If a marked change of the arm ratio is involved this type of rearrangement can be observed microscopically. There is also the possibility that more than one chromosome may be broken, in which case fusion may take place between non-homologous chromosomes. Some of the products of such rearrangements are shown in Figs. 20 - 25. Resulting chromosomes which contain only one centromere are perfectly stable, and behave normally at mitosis; other products such as ring chromosomes and those with more than one centromere may get involved in mechanical difficulties when they begin to separate to opposite poles. Thus this type of product is also gradually eliminated.

A great deal of investigation of the effects of radiation upon cell division and chromosomes has been performed on plant material, and on Drosophila. Various workers have

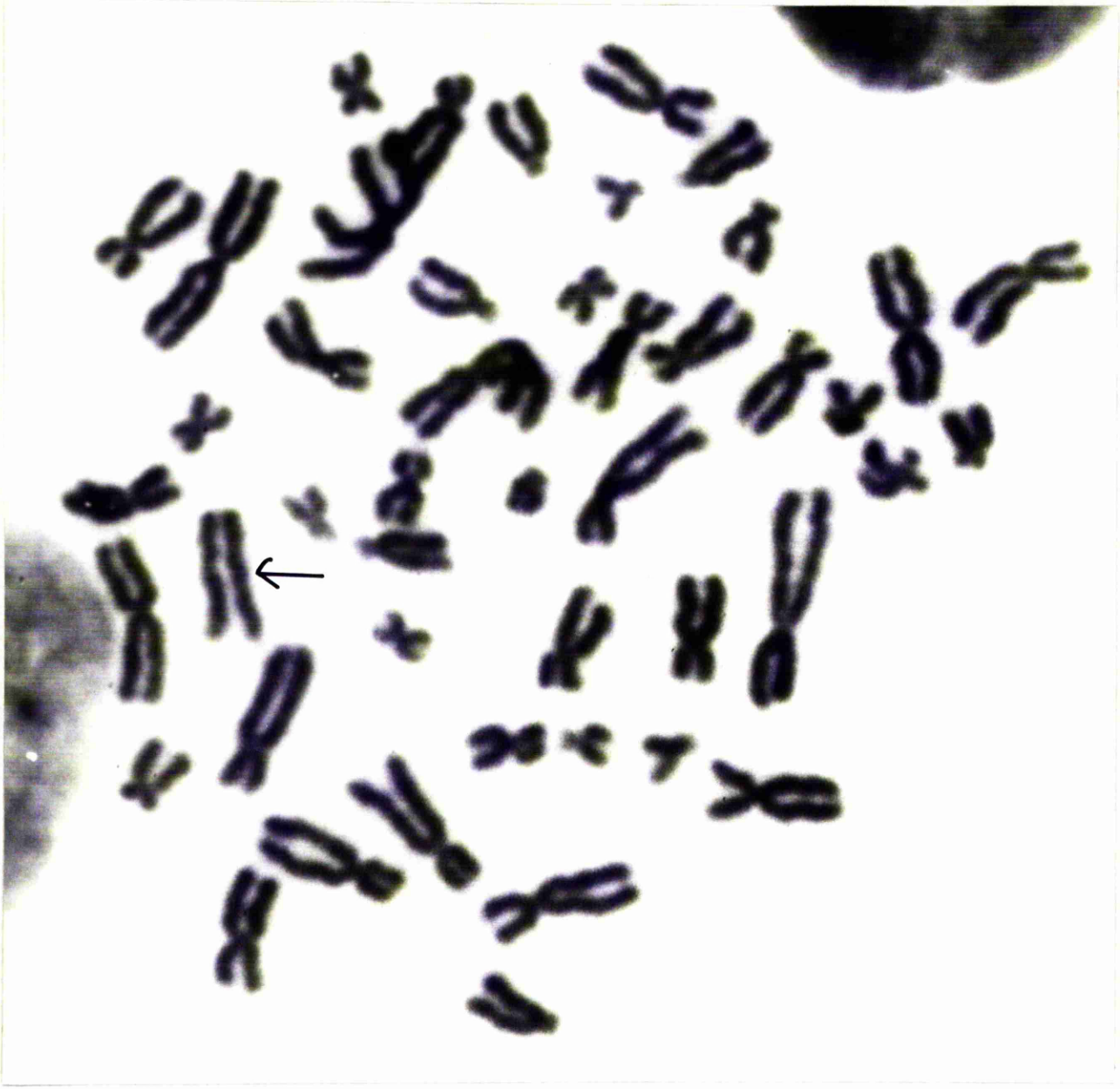


Figure 20. Irradiated cell showing an acentric fragment.

shown that the amount of damage produced is affected by the intensity and duration of the dose (Sax, 1939; Koller, 1952), by the stage of the nucleic acid cycle of the cell at time of irradiation (Swanson, 1942; Darlington and La Cour, 1944) and is not affected by variations in temperature (Darlington and La Cour, 1944). The frequency and distribution of the induced breaks has been investigated (Kaufmann and Demerec, 1937; Kaufmann, 1939), and dose-effect relationships have been established.

Only recently have studies on human material become possible (Bender, 1957; Bender and Gooch, 1961 a; Chu and Giles, 1959; Chu, Giles and Passano, 1961). It has been shown, using the classical tissue culture methods, that the effects are similar to those produced in irradiation experiments on other material, and dose frequency relationships have been explored. The peripheral blood method has been used in two different ways: (1) effect of irradiation of cells *in vitro* has been studied (Bell and Baker, 1962; Bender and Gooch, 1962 a); (2) effect on the chromosomes of the cells of the peripheral blood when the individual has been irradiated, either by accident (Bender and Gooch, 1962 b) or in the course of treatment (Tough *et al.*, 1960; Boyd,

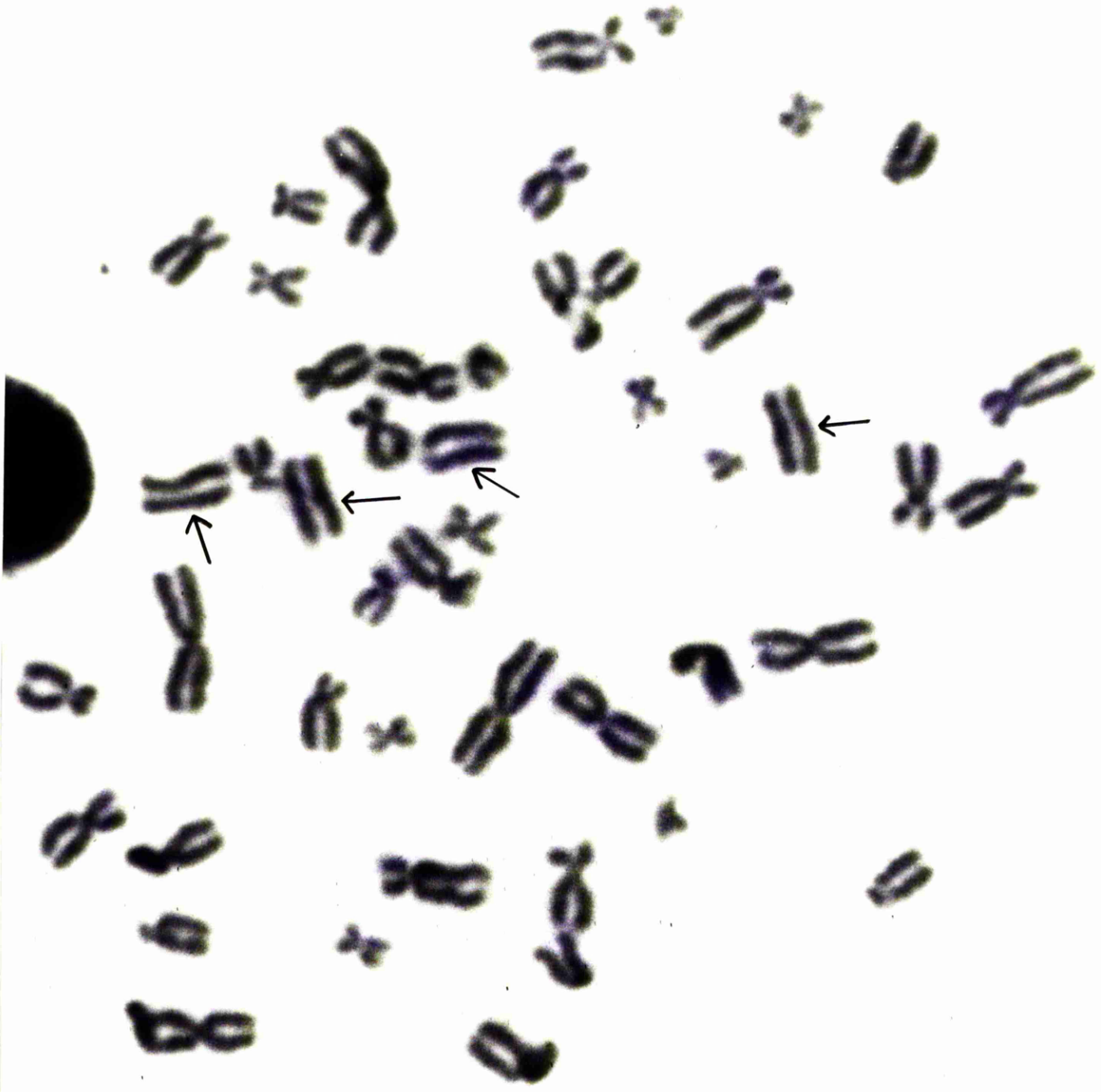


Figure 21. Irradiated cell showing four acentric fragments.

Buchanan and Lennox, 1961; Buckton et al., 1962; Conen, Bell and Aspin, 1963) has been studied.

In view of the X-ray induced chromosome damage reported by Tough et al. in the peripheral blood cells of individuals being irradiated for ankylosing spondylitis, it was decided that this author should investigate to what extent similar aberrations were to be found in the cells of patients receiving ^{131}I therapy for thyroid disease.

Present Material

(a) Note on ^{131}I

The radiation from ^{131}I consists of β particles and γ rays. In the case of β particles (fast electrons) the dose is essentially confined to the region containing the ^{131}I since their range in tissue is only a few mm.; γ rays, however, (electromagnetic radiation of the same sort as X-rays), are much more penetrating and absorption is rarely complete within the tissue containing them. The half-life, i.e. the time in which the radioactivity decreases by half, is eight days.

^{131}I is administered to the patient by mouth, and is rapidly absorbed into the blood stream, where it is at its maximum concentration one hour after the administration of the dose. During the time it is circulating in the blood the

Figure 22. Irradiated cell showing an abnormally long acrocentric chromosome.

patient is subject to irradiation throughout the whole body. It is selectively absorbed by the thyroid, and only very small amounts are taken up by other organs; for this reason it is used quite extensively in the treatment of thyroid disease in man. It is fairly quickly excreted via the kidneys, three-quarters of the given dose being lost within two days.

(b) Patients studied

The patients studied fell into three groups. In the first place, five individuals who had received a tracer dose of 25 μc. had a blood sample withdrawn for chromosome studies three days after the dose had been administered.

The second group of patients were all middle-aged subjects being treated for thyrotoxicosis by the method of Crooks et al. (1960). Four of these patients were included in a preliminary account of this study (Boyd, Buchanan and Lennox, 1961). They were given doses ranging from 5 to 8 mc. Blood samples withdrawn before the dose was administered acted as controls, and further samples were taken 3 days and/or 5 days after treatment. In only one case had there been any previous treatment with ^{131}I. This was patient No. 172, one of the two patients receiving a 7 mc. dose, who had received a dose of 7 mc. nine months before the relevant

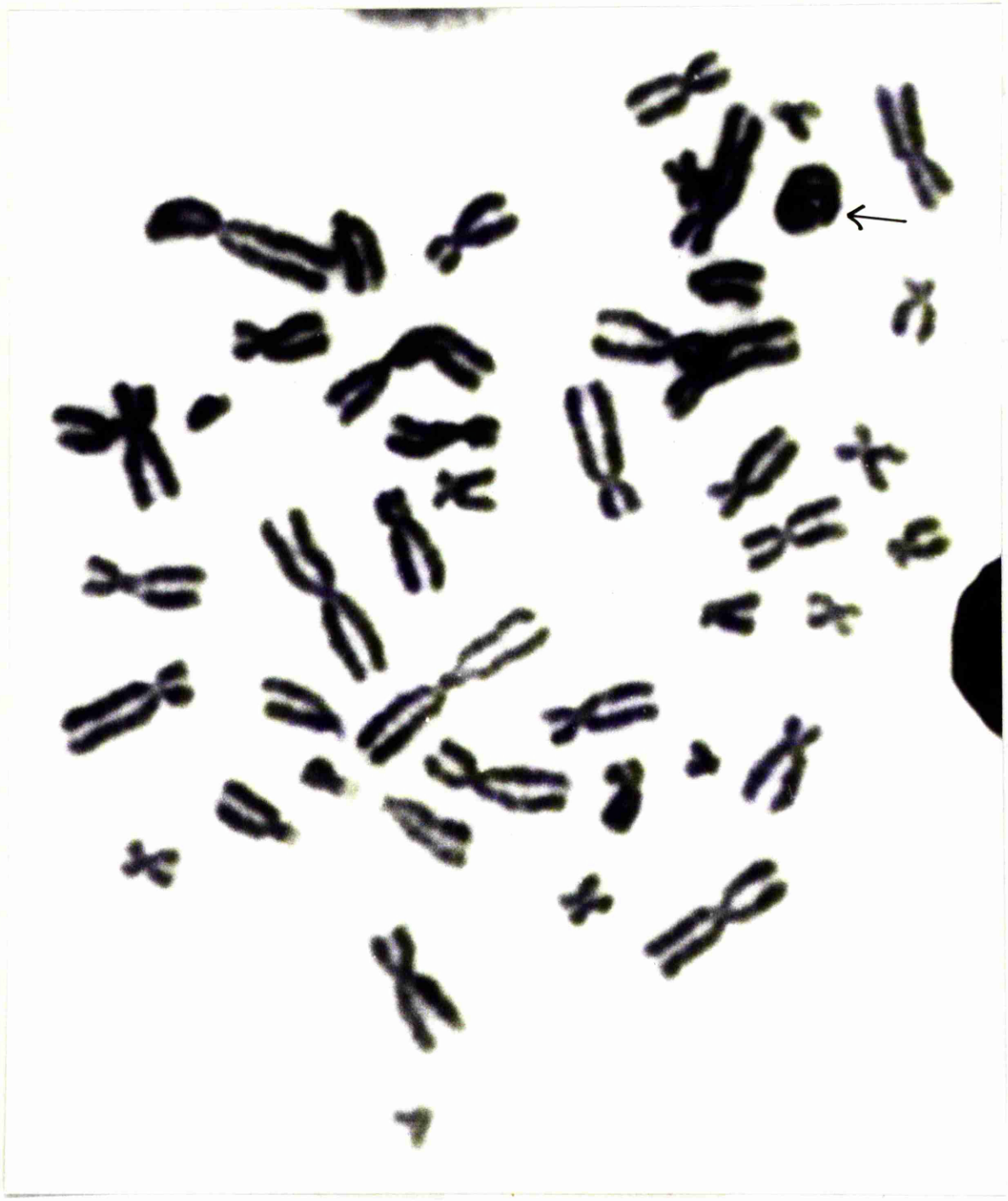


Figure 23. Irradiated cell showing a ring chromosome.

treatment, and a similar dose of 7 mc. seven months previous to that. One patient in this group was again available for study five months after treatment, and a second individual was studied one year after administration of the dose.

The third group consists of four patients who received a higher dose. Two individuals with thyroid malignancy were given 25 and 50 mc. respectively; neither had had any previous ^{131}I therapy. The two remaining cases (cases 5 and 6 of published paper) were both females aged 67 years. Patient No. 90 had thyroid adenocarcinoma excised in 1953. She had a dose of 80 mc. in December 1957, and five later doses of 100 mc. each in February, May, and October 1958, and February 1960. The dose with which this report is concerned, 150 mc., was given in November 1960. Patient No. 104 had adenocarcinoma excised in 1946. 3000 r. of deep X-rays given to lumbar spine in 1948. She received an ablative dose of 100 mc. in March 1960, and another in July. The relevant dose in the present study, also 100 mc., was given in January 1961. Again, samples withdrawn before the dose was administered acted as controls, and other samples were withdrawn at various intervals after treatment.

Figure 24. Irradiated cell showing dicentric chromosome.

The mitoses studied here are the first division of the leukocytes in culture, but since in most cases an interval of 3-5 days had elapsed between administration of the dose and withdrawal of the sample, some at least of the cells will have divided in the body. The samples thus contain a very mixed population of cells, some of which have divided since the irradiation while others have not; in addition, some would be irradiated in the single strand state, while others would already have doubled. No quantitative studies are therefore possible in this system.

In order to obtain a more quantitative evaluation of the effect produced on the chromosomes by the ^{131}I, an *in vitro* experiment was carried out. Since there is some evidence (Revell, 1952; Kaufmann, 1939) that heterochromatic regions of the chromosomes are more susceptible to breakage by irradiation, this writer also wished to investigate whether any particular chromosome, or chromosome region, was preferentially involved. In this experiment, a sample of peripheral blood was withdrawn from a non-irradiated individual and a series of identical cultures was set up. A solution containing ^{131}I was then added to the cultures to deliver a given dose to them during the three day culture

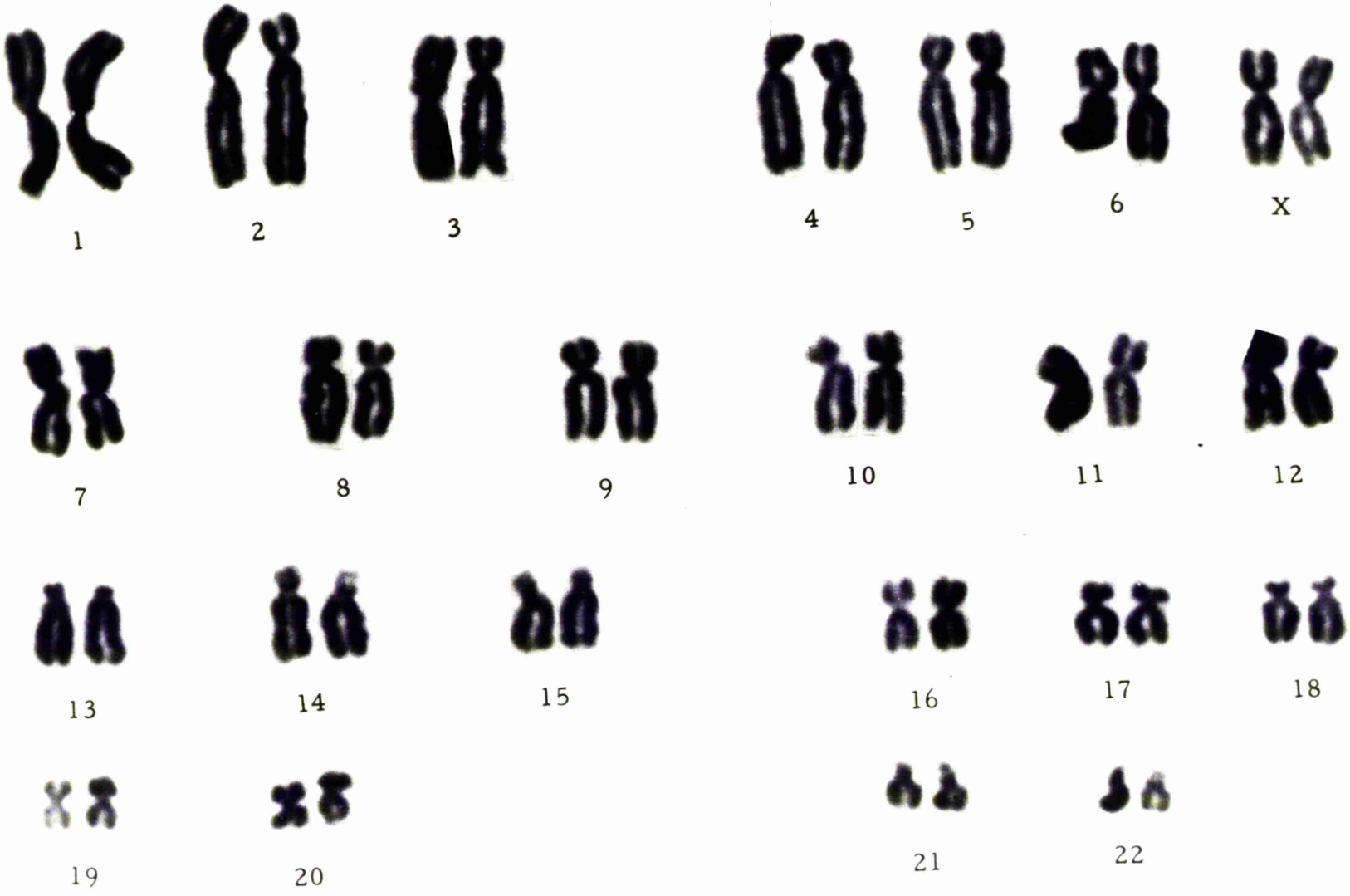


Figure 25. Irradiated cell showing rearrangements in a chromosome No. 2 and a chromosome No. 3.

period. One of the cultures was left untreated as a control. The cells were harvested and processed in the usual way. These cells are thus examined in their first post-irradiation mitosis, although here again, since the irradiation continued over the three day period, chromosomes would be irradiated in both the split and unsplit state.

Method of Scoring Cells

In this section each cell scored was treated as follows. First the number of chromosomes present was ascertained, and then the chromosomes were examined for aberrations by determining microscopically whether the distribution into subgroups was normal. By this method most of the rearrangements which alter the morphology of the chromosome can be recognised, though with the present limits of resolution many rearrangements will not be obvious. Where there was any ambiguity, photographs were taken and karyotypes constructed. A total of just over 3,000 cells was studied.

Method of Recording Aberrations

This was based on the classification used by the Edinburgh group in their report (Buckton et al., 1962), but with some important modifications.

Type A cells. These are cells which have no evidence of structural abnormality, although they may have more or less than the modal number of chromosomes.

Type B cells. These are cells whose chromosomes are apparently normal except for the presence of a chromatid break. Only cells where there is definite evidence, such as displacement of the distal fragment, that a break in the chromatid has taken place are included; unlike the practice of the Edinburgh group, those with chromatid gaps and isolocus gaps are not scored. (See Appendix D.)

Type C cells. These cells show evidence of structural rearrangement. They are sub-divided into two groups:

C_1 cells - these are the unstable types of rearrangement, ring chromosomes, di- or multi-centrics, and acentric fragments, all of which are liable to be lost in cell division.

C_2 cells - these are the stable rearrangements which have only one centromere, and can perpetuate themselves. Cells in which the chromosomes do not

resemble members of the normal set but are very conspicuous, and cells in which the chromosomes are indistinguishable from the normal but the division into sub-groups is abnormal, have both been included in the one group here and not sub-divided. Both types probably arise in the same way, the only difference between them being the difficulty of recognising the second type. It is certain that many cells carrying C_2 rearrangements are not visible microscopically with the techniques available at present. Hence this figure will always represent the minimum number of stable rearrangements present.

Results

Tracer doses

The figures are given in Table XVIII and in Fig. 26. It was unfortunately not possible to obtain pre-dose samples from the patients receiving tracer doses to use as controls. The mean of the control figures of the patients receiving low doses of ^{131}I was therefore used as a control figure; any deviation from normal due to the thyrotoxicosis should thus be taken into account. There was a great deal of variation in the percentage of modal cells present in the blood withdrawn three days after administration of the dose, but the average of these figures shows a drop of 4% from the modal.

This is accounted for by a very slight increase both of the non-modal 'A' cells and cells with chromosome rearrangements. In all this effect is very slight.

TABLE XVIII

Tracer doses

Ref. No.	% cells with chromosome no. 45	46	47	>47	% cells with aberrations B	C_1	C_2	Total cells counted
Control	7	90	2	-	-	1	0·5	258
127	4	92	2	-	-	2	-	48
165	9	76	6·5	-	2	-	6·5	46
168	5	87	3	-	-	5	-	38
169	5	92	1	-	-	1	-	74
170	13	82	3	-	-	-	2	61
Average	7·5	86	3		0·5	1·5	1·5	267

Low doses

On the whole growth in this series of cultures was disappointing and the number of cells available for counting was consequently limited. The actual percentage of cells in each class varies from patient to patient, but the general trend is the same. This variation could be due to a different

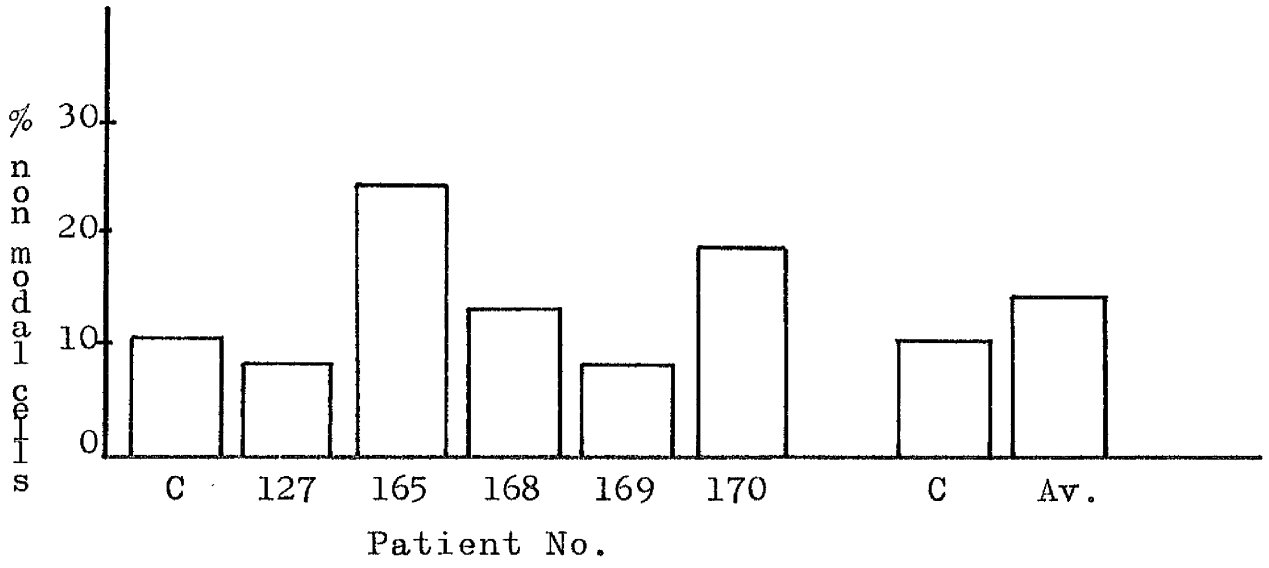


Figure 26. Percentage of non-modal cells in five patients who had received tracer doses of ^{131}I.

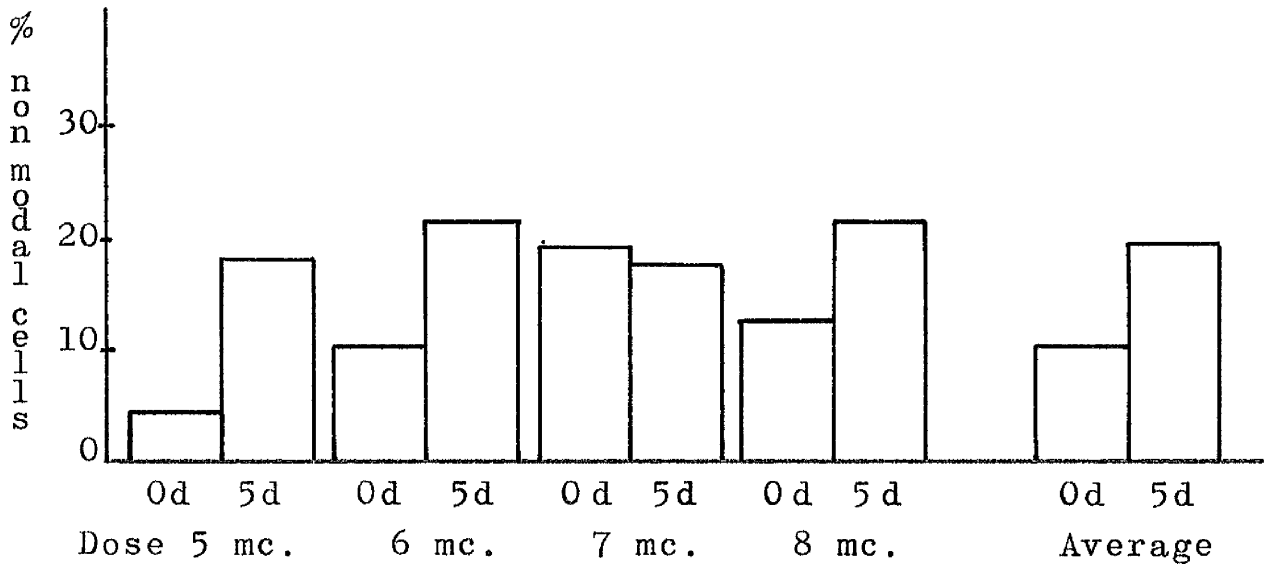


Figure 27. Comparison of non-modal cells before and 5 days after low doses of ^{131}I.

degree of response in each patient, or to sampling errors because the number of cells is small. Since most authors agree that tissues from different individuals show similar response (Chu, Giles and Passano, 1961), the latter is the more likely cause of the variation. Thus the counts from patients receiving the same dose have been summed. The figures are given in Table XIX and in Fig. 27. (The figures for the three patients receiving 8 mc. of ^{131}I are given in full in Appendix E.)

TABLE XIX

Low doses

No. of patients	Dose mc.	Time after dose	% cells with chromosome types: A 45	A 46	A 47	A >47	B	C_1	C_2	Total cell count
2	5	0	3·5	96·5	-	-	-	-	-	57
		5 days	11	81	2	-	-	2	4	85
3	6	0	6	90	4	-	-	-	-	53
		5 days	13	79	4	-	-	-	4	47
		5 months	2	92	2	-	1	-	2	83
		1 year	5	88	-	-	-	2	5	42
2	7	0	10·5	81·5	3	-	-	3	3	38
		5 days	8	83	2	1	1	1	2	84
3	8	0	9	88	2	-	-	1	-	110
		3 days	10	79	4	2	-	3	2	122
		5 days	10	79	2	1	-	5	3	114
TOTAL	5-8 mc.	C	7	90	2	-	-	1	0·5	(258)
		5 days	10	81	2	0·5	0·5	3	3	(330)

C = control

In general, a therapeutic dose of 5-8 mc. depresses the modal cells by about 9%. As in the case of tracer doses, non-modal 'A' cells and cells with chromosomal rearrangements are increased. This effect is also small but it is of interest to note that both stable and unstable chromosome rearrangements are still present 1 year after administration of as low a dose as 6 mc.

High doses

The figures are given in Table XX and Fig. 28. Unfortunately not all the cultures initiated were successful, so the data are not as full as had been hoped. Since in two cases the control cultures failed to grow, this cannot be entirely due to the irradiation. However, it was noticeable that cultures set up within the first three days after the dose were disappointing, both in number of mitoses and in cytological quality.

The data from the patient given 50 mc. indicate that blood withdrawn as early as 6 hours after administration of the dose shows moderate deviation from normal; this effect is increased in blood withdrawn 24 hours after administration of the dose.

TABLE XX

High doses

Dose mc.	Time after dose	% cells with chromosome type A: 45	46	47	>47	B	C_1	C_2	Total cells counted	Fall in modal cells	Rise in C type cells
25	0	--	--	--	--	--	--	--	--	--	--
	5 days	10	76	1	1	2	1	9	100	11	10
50	0	10	87	3	--	--	--	--	31	--	--
	6 hrs	12	78	2·5	4	--	1	2·5	81	9	3·5
	24 hrs	18·5	68·5	4	--	--	4	5·5	54	19	9·5
100	0	6	90	--	--	--	3	--	31	--	--
	24 hrs	30	59	--	--	--	4	7	27	31	8
	48 "	14	55	--	9	--	18	4	22	35	19*
	72 "	17	70	6	--	--	4	4	83	20	5
	1 week	15	72·5	2·5	--	--	5	5	80	18	7
	1 year	19	71	2	--	--	6	2	96	19	5
150	0	--	--	--	--	--	--	--	--	--	--
	5 days	8	70	8	5	1	4	3	73	20	4
	6 months	5	79	2	--	3	8	2	95	11	7

* largely due to unstable forms

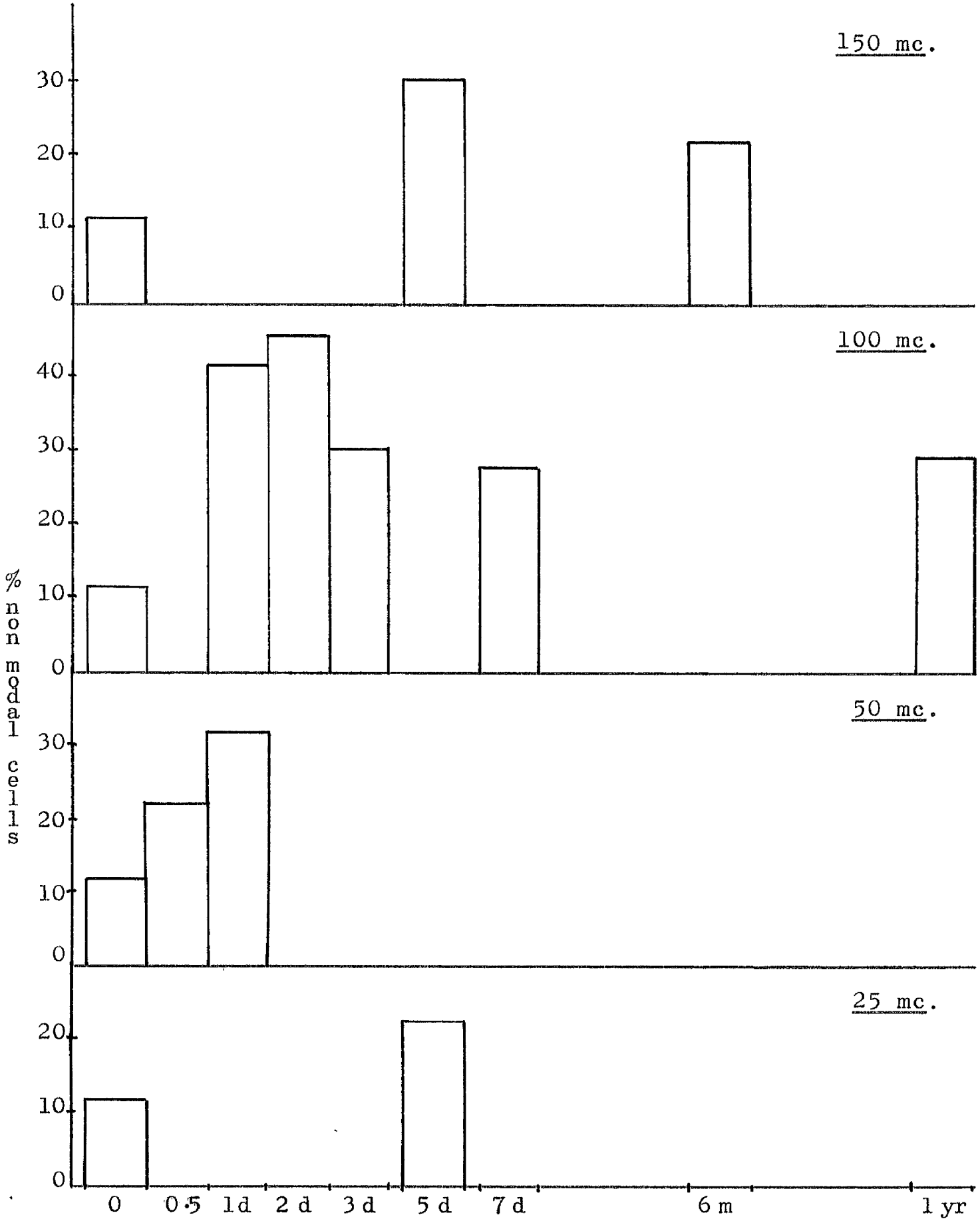


Figure 28. Percentage of non-modal cells in four patients who had received high doses of ^{131}I.

The data from the patient given 100 mc. indicate that the maximum deviation from normal is shown in blood withdrawn 48 hours after treatment, at which time a large number of chromosome rearrangements are present. Thereafter it becomes more normal, although chromosome rearrangements are still increased 1 year after the dose. The presence of 19% of cells with 45 chromosomes at this time is surprising, since 5 months after a dose of 150 mc. this figure had returned to normal.

It must be pointed out that the number of cells counted in each of the three groups of patients described here was small, and so the figures may not be an accurate measure of the effect produced. They do suggest, however, that there is a consistent tendency for therapeutic doses of ^{131}I to increase the proportion of abnormal cells in the peripheral blood. Larger scale studies are necessary before this effect can be quantitatively evaluated.

In vitro experiments

The total figures are given in Table XXI and Fig. 29. The individual figures for each of the three experiments involved are given in Appendix F. Non-modal 'A' cells and, more markedly, both types of 'C' cells, increased with the dose.

It was also noticeable that doses of 500-1000 r. resulted in poor yield of mitoses, and poor quality of those observed.

TABLE XXI

In vitro experiments

Dose	% cells with chromosome types: A				B	C_1	C_2	Total cells counted	Fall in modal cells	Rise in C type cells
	45	46	47	>47						
0	4	94	0·5	-	1	0·5	-	320	-	-
10r.	6	87	0·5	-	2	2	2·5	265	7	4
50r.	5	80	0·5	-	4	7	3·5	227	14	10
100r.	7	73	1	1	5	8	5	320	21	12·5
500r.	12	50	4	2	6	16	10	82	44	25·5
1000r.	6	35	-	6	6	35	12	17	59	46·5

It was attempted to discover whether chromosomes were involved randomly in rearrangements, or whether any particular members of the set were preferred. Karyotypes were made from 90 cells showing structural aberrations; the only selection involved was that the cells were suitable for karyotyping. All the chromosomes missing, or obviously involved in

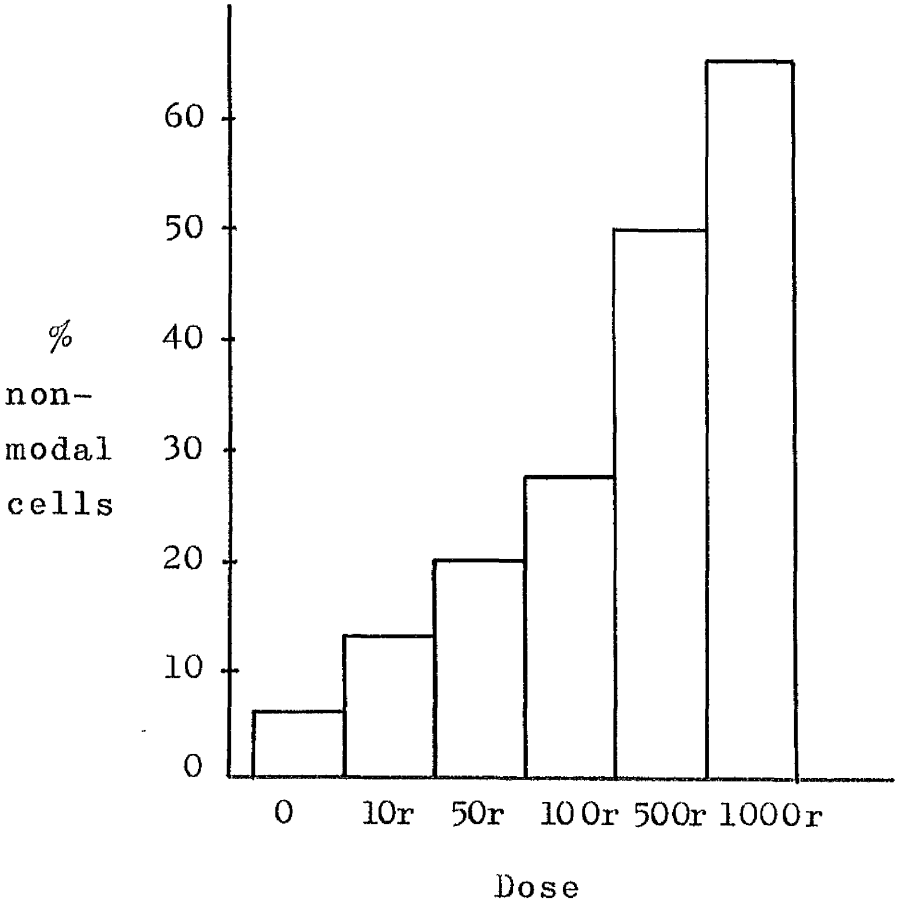


Figure 29. Percentage non-modal cells in cultures exposed to ^{131}I *in vitro*.

rearrangements, were recorded, and their distribution among the various sub-groups was compared with the expected distribution calculated from the relative lengths of the various chromosomes in each group (see Table XXII).

TABLE XXII

Chromosome group	1-3	4-5	6-X	7-12	13-15	16-18	19-20	21-22-Y
Expected	39	20	18	45	17	15	8	6
Found	32	20	20	49	12	13	8	8

It was noticeable that 15 chromosomes had been broken at the centromere, and 10 deleted chromosomes were observed where the break had taken place at the region of a secondary constriction.

Discussion

Green et al. (1961) have calculated that a dose of 5 mc. to a patient with thyrotoxicosis delivers a dose to the blood of 8·7 rads. The dose to the marrow is 80% of this, and since, as Buckton et al. point out, it is likely that damage observed in cells from blood cultures largely reflects the

effect of radiation on lymphopoietic tissue, this figure may be the more important. The dose to blood and marrow in patients receiving 5-8 mc. ^{131}I is thus of roughly the same order as the 10 rad. *in vitro* dose.

Non-modal 'A' cells

As shown in Tables XIX and XX, cultures prepared from patients after irradiation contain an increased percentage of these cells. There does not appear to be a correlation between the size of the increase and the dose to the patient.

A similar increase in non-modal 'A' cells was shown in the irradiated patients discussed by Buckton *et al*. (1962), and as in the present case the majority of these cells were hypodiploid. It would seem that loss of a chromosome during handling of the cells, caused by increased fragility of the cell membrane, could account for some of these hypodiploid cells. However, since irradiation is known to increase non-disjunction in Drosophila (Mavor, 1924), errors of cell division may also be involved.

The patient given 100 mc. ^{131}I shows a striking increase in hypodiploid cells and - atypically - this is apparently still present 1 year later. The reason for this is not clear; it is possible that this dose, combined with the

considerable radiation she had already received, has produced some more permanent effect.

The production of non-modal 'A' cells *in vivo* is in contrast with the *in vitro* figures, where this effect is slight. Similarly, *in vitro* experiments described by Bender and Gooch (1962 a) show no increase in cells with abnormal chromosome numbers.

Structural Aberrations

The *in vitro* data show that breakage and rearrangement increase as the dose of irradiation increases (as has been shown by many other workers). These cells are observed in their first post-irradiation mitosis, which is essential for assessing structural damage quantitatively; later, elimination of cells with unstable rearrangements or with unbalanced translocations which result in loss of chromosome material will have occurred. It is therefore not surprising that the *in vivo* data fluctuate. Although the total number of breaks produced by a given dose will be constant, the survival of cells carrying them will depend on the nature of the subsequent rearrangements the broken ends undergo.

The data in Table XXII show that the breaks produced are distributed randomly throughout the chromosome set, no

particular member being particularly susceptible. This is in agreement with the work of Oehlkers (1952) and of Ford (1949), who found that breaks produced by X and γ rays were random. There has been some suggestion that heterochromatic regions are more often involved (Kaufmann, 1939; Revell, 1952). In the present study, only 10 of the 168 chromosomes identified as taking part in rearrangements could be definitely said to have broken at secondary constriction regions. On the other hand, in many instances it is not possible to say exactly where the break has occurred, so that this is a minimum figure. This contrasts with the preferential breakage of these regions by chemicals (Oehlkers, 1952) and by virus infection (Moorhead and Saksela, 1963).

The presence of 15 cells with centric breakage is of interest. Such breakage is very occasionally seen in untreated cells, and Koller (1952) has shown that misdivision of centromere, and centric breakage of chromatids, are frequently seen in pollen grains after low intensity radiation.

One of the main objects in studying the effect of irradiation on human material is investigation of the hazards of ionising radiation in man. It is of interest from this point of view that after periods of 6 months to 1 year have

elapsed since therapeutic doses of ^{131}I, cells with chromosome rearrangements are still seen in the peripheral blood.

Buckton reports that the proportion of cells carrying stable rearrangements remains unchanged even 20 years after irradiation for ankylosing spondylitis, although unstable types decrease. Bender and Gooch (1962 b) described persistent chromosome aberrations, including unstable types such as ring chromosomes and dicentrics, 29 months after accidental exposure to radiation. In the present study more of this type of rearrangement than the stable type was observed 6 months to 1 year after high doses.

Pochin (1960) has concluded that there is no definite evidence of an increase in the number of cases of leukaemia in patients treated in this way as compared with the general population. However, Means, DeGroot and Stanbury point out that a disconcertingly high proportion of those who did develop the disease following ^{131}I had the acute variety. Baikie et al. (1961) found definite chromosomal abnormalities in 5 out of 7 cases of acute leukaemia following therapeutic irradiation. Buckton et al. (1962), on the other hand, point out that there is a 10-fold increase in the incidence of leukaemia among their patients being irradiated for ankylosing spondylitis.

Means, DeGroot and Stanbury also point out that fewer thyroid tumours have appeared in adults than might have been expected, although three children have developed suspicious nodules following ^{131}I for thyrotoxicosis. Thus there is no concrete evidence of an increased risk of leukaemia or thyroid tumour risk following ^{131}I therapy. The risk of increased mutation in germ cells which arises in patients of child-bearing age is also considered by the above authors to be small. This is an effect which cannot be measured directly; it must be considered in terms of the community rather than the individual. Volpe et al. (1961) quote Bercy as saying that a dose of 10 mc. ^{131}I gives a dose of only 1·25 rads to the ovaries. Indeed many authors are at pains to point out that the total body dose from 10 mc. ^{131}I (which Volpe quotes Merliss as estimating to be 7 rads) is similar to that received from a series of diagnostic X-rays. Yet it is sufficient to produce a degree of damage to the chromosomes of the cells circulating in the peripheral blood.

Conclusion

Treatment of patients with ^{131}I for thyroid disease appears to be accompanied by some production of chromosomal

aberrations in the cells of the peripheral blood, and these cells can remain for at least 6 months to 1 year. With the exception of the centromere effect typical of radiation at low intensities, no particular chromosome or chromosome region appears to be involved more than any other. There is no convincing evidence at present to connect these observed aberrations with an increased risk of leukaemia or thyroid tumour formation after treatment with ^{131}I.

CONCLUSION

The first object of the work described in this thesis was to establish the techniques involved in the study of human chromosomes from samples of bone marrow and peripheral blood. When this was achieved, the methods were applied to the investigation of the chromosome complement in various human conditions. These came into four main categories.

Mongolism

One of the most valuable applications of chromosome studies has been in the field of mongolism. Where the clinical diagnosis is in doubt, for example, it is important to investigate the presence or absence of an additional chromosome 21. Also, when a mongol child is born to young parents, chromosome studies reveal whether or not a translocated chromosome is being inherited and hence are of value in advising parents about the risk of a second affected child. This aspect of chromosome studies is rapidly becoming a routine hospital procedure.

The chromosomes of twenty young mothers of mongols have been investigated in this study, together with those of the father and/or the mongol child. Only one trans-

location mongol was found among this series, and since the karyotypes of both the parents were normal, this translocation had probably arisen spontaneously. This suggests that the incidence of inherited translocations in mongols born to young mothers, where no other relatives are affected, is relatively low.

Among this series of young mothers was one who was found to have, in addition to a normal diploid cell-line, a small proportion of cells trisomic for chromosome 21. Similar mosaicism has been reported by several authors. Thus in a small proportion of cases a mongol birth may be caused by mosaicism in the mother.

Sex chromosomes

Similarly, in an individual with abnormal sexual development, knowledge of the sex chromosome complement may be instructive, particularly if the patient is young. Chromosome studies have been applied in this way during the present work to nine cases or doubtful cases of Turner's syndrome. Of these, two proved to be mosaics of two cell-lines; in one case the sex chromosome complement was XO/XX, and in the other there was an XO cell-line and a cell-line with two X chromosomes, one of which was an isochromosome for the long arm of the X.

Other studies in the field of sex chromosome anomalies discovered four adult cases of the then newly described XXX syndrome. Investigation of 15 patients with Klinefelter's syndrome revealed 3 individuals with an unusual chromosome constitution: they had respectively an XX, an XXY/XXXY mosaic and an XXXXY sex chromosome complement. Similar sex chromosome constitutions have been described by other authors. As in all cases of human chromosome abnormalities, affected individuals are rare among the general population, and it is necessary to combine the information obtained by various groups in order to achieve an overall picture.

Various pathological conditions

In the course of this work chromosome studies were carried out on 43 patients with various pathological conditions, in which it was thought that an atypical chromosome pattern might be present. Of these, (i) 7 came into the category of genetically determined conditions and 6 had other distinct syndromes. All the individuals in these two groups had apparently normal chromosomes. Some of these conditions have also been studied by other authors, and apart from some minor deviations which have not proved to be consistent, the same result has been obtained.

(ii) 7 were parents of anencephalic foetuses; these proved to have normal chromosomes.

(iii) 9 patients studied had multiple congenital anomalies. With the exception of one case of the E trisomy syndrome, all the individuals in this group also had normal karyotypes. Many cases of multiple congenital anomalies have been studied by other authors; some have proved to have normal chromosomes and some have had an abnormality present. The latter are in the minority and are probably sporadic events, since there is no consistency in the chromosome anomaly involved, or the physical symptoms present. So far (apart from mongolism) only the three distinct syndromes - the D and E trisomy syndromes and the 'cri du chat' - have been associated with a consistent abnormality of the karyotype.

(iv) 14 patients had diseases of the blood. These also proved to have normal karyotypes, with one exception - an atypical case of chronic myeloid leukaemia. Chronic myeloid leukaemia is the only form of leukaemia so far found to be associated with a consistent chromosome abnormality.

Radiation

Finally, chromosome studies were carried out on 14 patients receiving therapeutic doses of ^{131}I and these

investigations suggested that this treatment was accompanied by the production of a proportion of abnormal cells in the peripheral blood. Many workers have studied irradiation effects in various ways. In view of the increase at present in man-made ionising radiation, this is a field in which much future study is desirable.

Future aspects

Widespread chromosome investigation of abnormal individuals is still necessary in the future. The study of short-lived infants has already led to the discovery of the D and E trisomy syndromes and may lead to other such discoveries; chromosome studies of spontaneous abortions and stillbirths may also be of value. A large-scale investigation of the variations to be found in the general population will be rewarding and informative. In other fields, such as the sex chromosome abnormalities, and in congenital anomalies, the fullest possible information is necessary. It is also likely that in the fields of cancer and leukaemia, chromosome studies will continue to be of value.

Improvements in technique are necessary in the future. The application of electron microscopy techniques to human

chromosomes will allow more detailed studies; improvement in methods for studying meiotic material will permit valuable pairing studies in trisomic or translocation-carrying males. Extension of the recent autoradiographic methods is also likely and may lead to investigations at molecular levels.

In instances where a chromosome abnormality is already established, family studies will provide valuable information about mechanism and frequency of non-disjunction, or translocation. The inheritance of translocations can be studied. Such investigations also make possible the search for evidence of transmission of autosomal genes, which in the case of mongolism has already begun. Thus important advances in various aspects of human chromosome investigation are likely in the future.

APPENDIX A

Reagents used in the tissue culture methods described in Section I were as follows.

A. Those obtained commercially

(1) Ringers solution* - made in tablet form by Oxoid

Oxoid Laboratory Preparations,
Thames House,
Queen Street Place, LONDON, E.C.4.

(2) Heparin - Pularin

Evans Medical Ltd.,
Speke, LIVERPOOL, England.

(3) Colcemid - available as 1 mg. tablets from

Ciba Laboratories Ltd.,
HORSHAM, Sussex.

(4) Cellosolve

The British Drug Houses Ltd.,
BDH Laboratory Chemicals Group,
POOLE, Dorset.

(5) Euparal + Euparal essence

Flatters and Garnett, Ltd.,
309 Oxford Road,
MANCHESTER, 13.

(6) Phytohaemagglutinin

Originally from Difco Laboratories,
DETROIT 1, Michigan, U.S.A.

Then from The Wellcome Research Laboratories,
BECKENHAM, England.

Both lots obtained dry in 5 ml. ampoules and made up in sterile doubly distilled water.

(7) T.C. 199* (Morgan, Morton and Parker)

Glaxo Laboratories Ltd.,
GREENFORD, Middlesex.

(8) Mounting media:

(a) 'DePeX' - available from G.T. Gurr Ltd.,
LONDON, S.W.6.

(b) Harleco Synthetic Medium

Hartman-Leddon Company,
PHILADELPHIA, Pa., U.S.A.

* Both these solutions can be made in the laboratory if preferred. Instructions for this are given in Paul, 1961.

B. Solutions prepared in the laboratory

(1) Hanks Balanced Salt Solution

See Paul, 1961, p. 81.

(2) Feulgen reagent and sulphurous acid rinse

Paul, 1961, p. 254.

(3) Glucose-saline solution (see text).

APPENDIX B

TABLE OF XXX FEMALES

Reference		Age	Sibship	Parental Age Mat.	Pat.	I.Q.	Sex chromatin 1 mass	2 masses	Chromosomes	External genitalia	Menstrual history	Height	Clinical features
Jacobs et al., 1959		35	3/3	41	40	normal	57%	14%	47/XXX	infantile	age 14–19 yrs then 2ndy amenorrhoea	5'9¼"	Breasts underdeveloped.
Jacobs et al., 1960		21	-	-	-	high grade M.D.	41%	48%	47/XXX	normal	normal	5'	Slightfflexion deformities.
de Carli et al., 1960		19	-	34	39	70	57%	8–16%	47/XXX	slightly under-developed	frequent and abundant	5'6"	Palpebral fissures slant slightly upwards from eye.
Sandberg, Crosswhite & Gordy, 1960		21	-	41	49	40	some show double sex chromatin		47/XXX	normal	regular from age 11	5'7"	Webbing of neck.
Stewart & Sanderson, 1960		35	4/5	42	42	70	38%	14%	47/XXX	normal	normal 5 children	5'6"	-
Johnston et al., 1961	1.	15	2/2	29	33	46	44%	40%	47/XXX	normal	normal	5'4½"	Mongoloid palms. ? Toxoplas-
	2.	26	1/1	17	33	<20	38%	50%	47/XXX	normal	irregular	5'4"	- mosis.
	3.	39	-	18	-	31	41%	37%	47/XXX	normal	normal	5'3¾"	-
Breg, Cornwall & Miller 1962	1.	14	-	19	-	70	-	-	47/XXX	normal	normal	-	Had a brother a mongol.
	2.	24	-	22	-	47	-	-	47/XXX	normal	normal	-	-
	3.	32	-	24	-	45	-	-	47/XXX	normal	normal 4 children (one has grand mal epilepsy)	-	Had 2 psychotic episodes. Sister with severe mental retardation. Cousin is trisomic mongol.
Hamerton, Jagiello & Kirman, 1962		23	2/3	21	27	low grade M.D.	22%	10%	47/XXX	normal	2 periods at 16 yrs	4'9"	Minimal breast development. Several contractures. Spasticity of all 4 extremities.
Aisters-Bauer, 1963		12	-	-	-	retarded	72%	12%	47/XXX	pre-pubertal female	-	-	Syndactyly & phocomelia digits, both hands and feet. Narrow palpebral fissures, repeated otitis media and tympanoplasties; speech defect.
Raphael, 1963		59	-	-	-	chronic schizophrenia	-	-	47/XXX	normal	normal 4 children and 2 miscarriages	5'8"	-
Ridler, Shapiro & McKibben 1963	1.	17	4/4	38	37	63	-	-	47/XXX	normal	slightly irregular	5'1"	Arachnodactyly with short little fingers.
	2.	17	-	-	-	subnormal	-	-	47/XXX	normal	normal	5'2"	Poorly developed breasts. Little fingers both hands markedly curved.
Close, 1964	1.	37	1/1	28	31	normal	-	15%	47/XXX	normal	normal 2 normal sons	5'7½"	-
	2.	42	3/3	41	47	normal	-	23%	47/XXX	normal	normal	5'8½"	-
Day, Larson & Wright, 1964	1.	-	4/4	24	29	61	35%	10%	47/XXX	-	normal 1 normal daught.	-	Systolic murmur. 2 sibs
	2.	39	1/7	19	32	30	49%	11%	47/XXX	normal	normal	short	delinquent, 3rd M.D. Cretinoid features.
	3.	-	1/3	18	19	35	29%	11%	47/XXX	normal	normal 1 normal son	-	-
Fraser et al., 1960	1.	30	4/8	28	-	50	43%	30%	47/XXX	normal	normal 1 normal son	5'1½"	-
Lennox Castle patients	2.	39	5/5	35	-	58	50%	25%	47/XXX	normal	irregular	5'9¼"	-
	3.	61	9/11	40+	-	38	35%	33%	47/XXX	normal	irregular	4'11½"	-
	4.	73	-	-	-	50	33%	30%	47/XXX	normal	unknown	5'1½"	-
Patient No. 5. present study		16	3/3	-	-	69	some show double sex chromatin		47/XXX	normal	normal	5'5½"	-
Carr, Barr & Plunkett 1961	1.	14	-	21	19	30	1, 2 and 3 sex chromatin masses present		48/XXXX	normal	normal	5'2"	Parents borderline I.Q. 2 maternal aunts M.D.
	2.	33	-	41	39	50			48/XXXX	normal	normal, though ovaries could not be felt.	5'4½"	No history of M.D.
Kesaree & Woolley 1963		16 months	4/5	22	26	severely retarded	1, 2, 3 and 4 sex chromatin masses present		49/XXXXX	normal	-	2'4"	Congenital malformations.

APPENDIX C

SOME XXXXX CASES

Reference	Age	Parental Age Mat.	Parental Age Pat.	I.Q.	Height	Buccal Smears 0	1	2	3	4	Genitalia	Testes	Skeleton	Other features
*Anders et al., 1960	8 years	29	29	21	-	-	52%	21%	9%	-	Hypoplastic scrotum	Bilateral cryptorchidism.	Microcephaly: bilateral synostosis of radius and ulna.	
Fraccaro, Kaijser, & Lindsten 1960	7 years	23	29	probably M.D.	-	6	18 out of 300	82	148	14	Scrotum underdeveloped & divided. No prostate palpable.	Pea shaped mass in each side of scrotum. Mostly connective tissue. A few seminif. tubules with undifferentiated cells.	Ossification centres of multangulum minus and capitulum radii were missing.	Flat occiput. Epicanthic eye folds. Protruding abdomen.
Miller et al., 1961	21 years	23	32	21	5'4½"	-	-	-	14%	-	Penis small.	Small scrotum with small mass 1 cm in diam. on L. but only chord-like structure on R.	Multiple abnormalities of skeletal system.	Cleft palate. Strabismus.
Case 1.	4⅔ years	23	24	20	3'3½"	-	24	40	30	-	Penis small.	Scrotum normal but testes not palpable.	Skull small. Bilateral cubitus valgus. Incurved 5th fingers and toes. Bone age retarded.	High arched palate. Divergent strabismus. Hypertelorism.
Barr et al., 1962 Case 2.	7½ months	38	38	mental age 4-5 months	2'2"	18	41	31	15	-	Penis very short.	Scrotum small. Testes small, palpable outside inguinal ring.	Small skull. Bilateral limitation of supination of forearms. Shaft of radius and ulna short and bowed. Bony fusion between upper radius and ulna.	
Fraccaro, Klinger & Schutt, 1962	12 years	26	29	35	4'10"	8	31	64	73	5	Penis small. Scrotum rudimentary.	No testes palpable.	Supination of both forearms restricted due to bony fixation of radius and ulna. Little fingers incurved. Lordosis.	Flat feet. Pot belly. Mild hypertelorism. Slight prognathos.
*Turpin et al., 1962	3 months	25	-	retarded development	-	-	6%	20%	some cells	-	Penis short.	Size of a pea.	Delayed bone age. Malformation of metacarpals.	Microcephaly. Small size and weight.
Atkins et al., 1963	21 months	39	23	-	2'6"	281 56%	154 31%	54 11%	11 2%	-	Penis and scrotum small.	Small but descended.	Retardation of bone maturation.	
Scherz & Roeckel 1963	17 months	24	25	mental age 5-6 months	-	8	7	37	48	-	Penis small.	Located high in underdeveloped scrotum.	Multiple skeletal defects.	Heart murmur. Palate high and arched, head dolichocephalic.
Case 1.	5 years	32	32	40	3'2"	33	29	27	11	-	Penis small; scrotum small and empty.	Could not be palpated in inguinal canals or perineum.	Incurved 5th fingers. Limitation of hand pronation and supination etc.	Abnormal facies. Downward slant of palpebral fissures. Wide set eyes.
Joseph, Anders & Taylor, 1964 Case 2.	9 years	28	30	50	4'7"	-	-	-	-	-	Penis small; scrotum flat.	Small soft mass in R. scrotum; L. empty.	Bilateral synostosis.	Ductus arteriosus. Wide set eyes, slanting downward, epicanthic folds. Neck short, moderate webbing.
Present study. Fraser et al., 1961	8½ years	22	24	mental age 2 yrs 8 mths	3'6"	24%	36%	30%	10%	-	Penis normal. Scrotum underdeveloped.	Undescended.	Multiple bony abnormalities.	Cleft palate.

* Anders, G., Prader, A., Hauschteck, E., Schärer, K., Siebenmann, R.E., and Heller, R. (1960) Helv. paediat. Acta, 15, 515.
** Turpin, R., Lafourcade, J., Cruveiller, J., Lejeune, J. (1962) Bull. Soc. Med. Hôpitaux de Paris, p. 916.

APPENDIX D

Note on Chromatid and Isochromatid Gaps

A chromatid gap is defined as a non-staining region in a chromatid; an isochromatid gap has non-staining regions in both chromatids at the same level.

The scoring and interpretation of these breaks is controversial. Some authors include them with chromatid breaks (Greenblatt, 1961; and with some qualification, Buckton *et al.*, 1962). Others put them in a class by themselves as achromatic lesions (Conen, Bell and Aspin, 1963; Bender and Gooch, 1963). The ambiguity of their classification was recognised by Chu, Giles and Passano (1961), who pointed out that their recognition depends, among other things, on the stage of cell division and in staining reactions. In this author's experience such lesions can in fact be seen in untreated material - a point also mentioned by Conen, Bell and Aspin (1963).

In the present study karyotypes were constructed in 30 relevant cells. 15 out of the 32 lesions of this type present coincided with the site of known secondary

constrictions (see Fig. 30). This surprisingly high proportion suggests the possibility that some of these lesions are in fact exaggerated secondary constriction regions. Such an effect has been described by Moorhead and Saksela (1963) in virus-induced chromosome aberrations.

They may on the other hand represent genuine breaks; radiomimetic chemicals are known preferentially to affect achromatic regions (Oehlkers, 1952; Revell, 1952), but there is little evidence of this in the case of ionising radiations. In fact there is recent evidence that these achromatic lesions do not represent true or complete breaks in the chromosome thread (Revell, 1957-58, 1959; Östergren and Wakonig, 1954). Consequently they have not been scored as breaks in the present study.

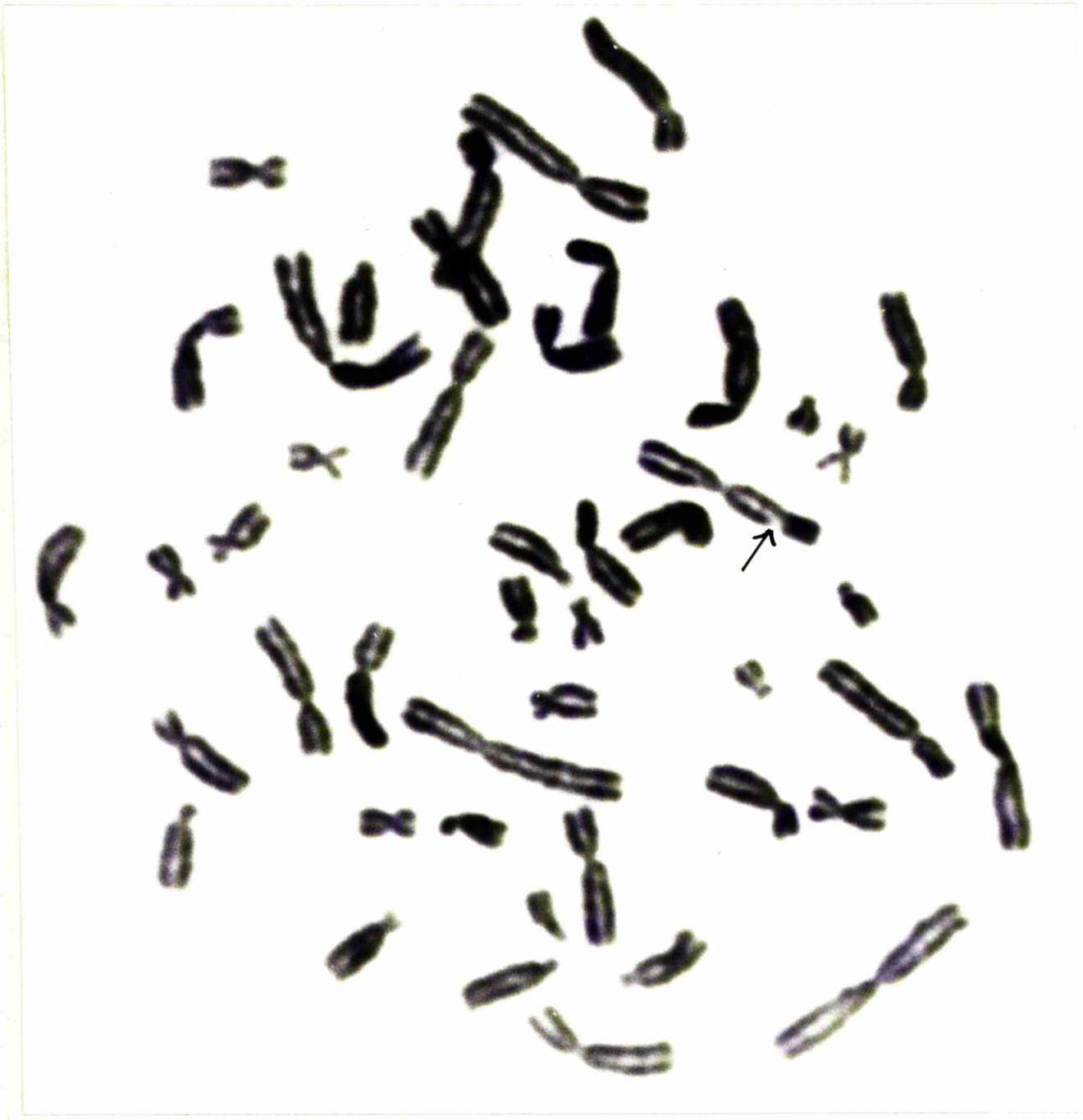


Figure 30. A chromatid gap at the secondary constriction region of chromosome No. 3.

APPENDIX E

Distribution of counts in three patients given 8 mc. ^{131}I

No.	Days after dose	% cells with chromosome types: A 45	A 46	A 47	A >47	B	C_1	C_2	Total cells counted
100	0	9	91	-	-	-	-	-	23
	3	11	77	8	-	-	4	-	26
	5	8	84	-	-	-	8	-	37
133	0	5	90	-	-	-	5	-	21
	3	15	75	3	-	-	-	6	32
	5	12·5	72·5	-	2·5	-	5	10	40
164	0	11	86	3	-	-	-	-	66
	3	6	84	3	1·5	-	5	-	64
	5	8	83	5	-	-	3	-	36

APPENDIX F

Chromosome counts in in vitro experiments with ^{131}I

Dose (r.)	% cells with chromosome types A: 45	46	47	>47	B	C_1	C_2	Total cells counted	Fall in modal cells	Rise in C type cells
0	2	97	1	-	-	-	-	100	-	-
10	11	82	-	-	1	1	4	93	15	5
50	4	84	-	-	3	7	3	124	13	10
100	8·5	70·5	-	-	4	12	4	94	27·5	16
500	12	48	2	4	6	14	14	50	53	28

Dose (r.)	% cells with chromosome types A: 45	46	47	>47	B	C_1	C_2	Total cells counted	Fall in modal cells	Rise in C type cells
0	3	93	0·5	-	1·5	1	-	140	-	-
10	5·5	86	-	-	3	1	3	91	13	3
50	4	77	1	-	5	8	5	77	16	12
100	4	77	-	-	5	6	8	100	16	13
500	12·5	53	6	-	6	19	3	32	40	21

Dose (r.)	% cells with chromosome types A: 45	46	47	>47	B	C_1	C_2	Total cells counted	Fall in modal cells	Rise in C type cells
0	9	90	-	-	1	-	-	80	-	-
10	2·5	90	2·5	-	1	4	-	81	-	4
50	11	81	-	-	4	4	-	26	9	4
100	9	71	2	2	5	8	3	126	19	11
1000	6	35	-	6	6	35	12	17	55	47

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