Leukaemia cytogenetics / H. Jackson Woodliff.

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LEUKAEMIA CYTOGENETICS

H. JACKSON WOODLIFF

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THE cause of most cases of leukaemia remains unknown and the discovery in the late 1950's of chromosomal abnormalities in cells from patients with this disorder opened up a new field of investigation. Now, after a decade of research by workers throughout the world, the subject is ripe for review.

In this monograph, the value of chromosome analysis in the diagnosis and management of patients with leukaemia and allied disorders is critically assessed. The contribution of such studies to aetiology and pathogenesis is considered and the findings in pre-leukaemia reviewed. The limitations of the usual techniques and the prospects for future studies are discussed.

In preparing this balanced account of the subject, the author has reviewed over 650 papers which are listed in the bibliography and considered the findings in the light of his own experience in the cytogenetic analysis of material from more than 200 patients.

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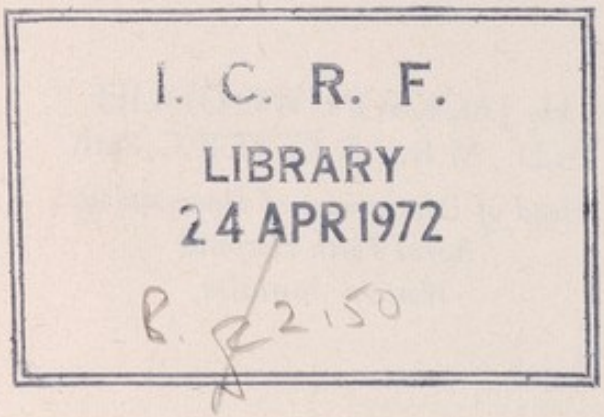

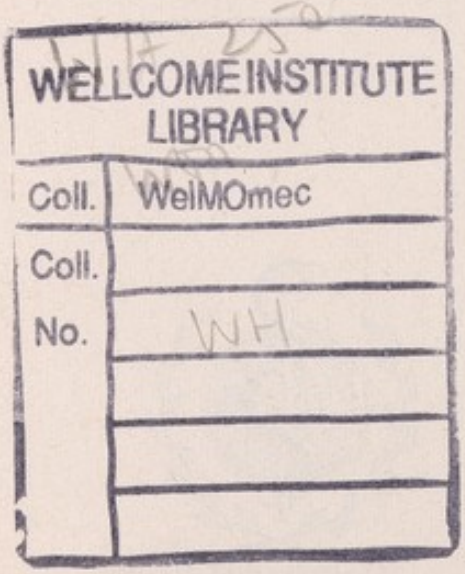

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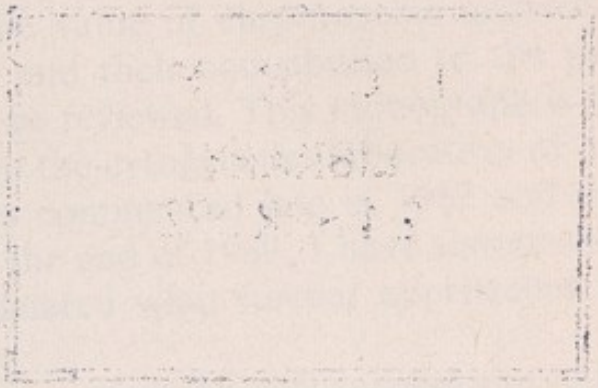
To my Mother

Frances Alice Woodliff



PREFACE

THE finding in 1958 of a chromosomal abnormality in cells from a patient with acute leukaemia stimulated further cytogenetic studies of the leukaemias and allied disorders. During the 1960s many research groups were active in this field and now after a decade of study the value of chromosome analyses in diagnosis and management, and their contribution to the pathogenesis of the disorders, can be reviewed. This monograph is based on eight years' experience in the cytogenetics laboratory of my department where such studies commenced late in 1962 and on reports and other literature to the end of 1969; I have summarised the present position and considered what further approaches might be profitable.

The abbreviations, symbols and conventions used in the text are those of the *British Journal of Haematology* (1970, **18,** 3-12) and the Chicago Conference (1966).

I am grateful to the many colleagues who have helped me in various ways:

Mr. P. Onesti, Dr. L. Dougan, Mrs. P. Stevenson, Mrs. G. Cohen, Mrs. L. Chipper, Mrs. W. Gallon and Mr. A. Leong, who worked in the cytogenetics laboratory for varying periods. Dr. D. G. Goodall and Mr. N. S. Stenhouse for statistical analyses.

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H. J. WOODLIFF

December, 1970

CONTENTS

Chapter I

INTRODUCTION

THE term "cytogenetics" is derived from the two parent sciences of cytology, the study of cells, and genetics, the study of transmission of biological information from one generation of organisms or cells to the next. Cytogenetics is largely concerned with the appearance and behaviour of chromosomes, as they are the constituents of the cell nuclei which carry the genes or units of inheritance. Both plant and animal chromosomes have been studied for many years but technical difficulties have prevented extensive studies of human material until comparatively recently. The number of human chromosomes had generally been thought to be forty-eight until 1956 when Tjio and Levan (1956) reported the true number to be forty-six (Chu, 1960). This was rapidly confirmed (Ford and Hamerton, 1956*a* and *b*; Bender, 1957; Hsu *et al.*, 1957) and the modern era of human cytogenetics began. Considerable advances have since been made and there is now a large literature dealing with chromosome abnormalities in congenital and neoplastic disorders.

The diseases considered here are the myeloproliferative and lymphoproliferative disorders, neoplastic proliferations of the haemopoietic and lymphoid tissues which include the leukaemias, lymphomas and their allied disorders; the names and possible inter-relationships are given in Fig. 1.

The first findings of a chromosomal abnormality in human leukaemia by Ford *et al.* in 1958, stimulated many others to undertake research into the subject. Since then a vast amount of information has been published and will be reviewed here. First, however, a brief history of the techniques which have been employed will be given; the methods will then be discussed in more detail, the normal human chromosome complement described and the types of abnormality which might be found considered. Subsequent chapters will deal with the different disorders.

Chromosomes are only apparent in cells whose nuclei are dividing by mitosis or meiosis. Mitosis is the nuclear division stage in the life cycle of individual proliferating somatic cells. Between

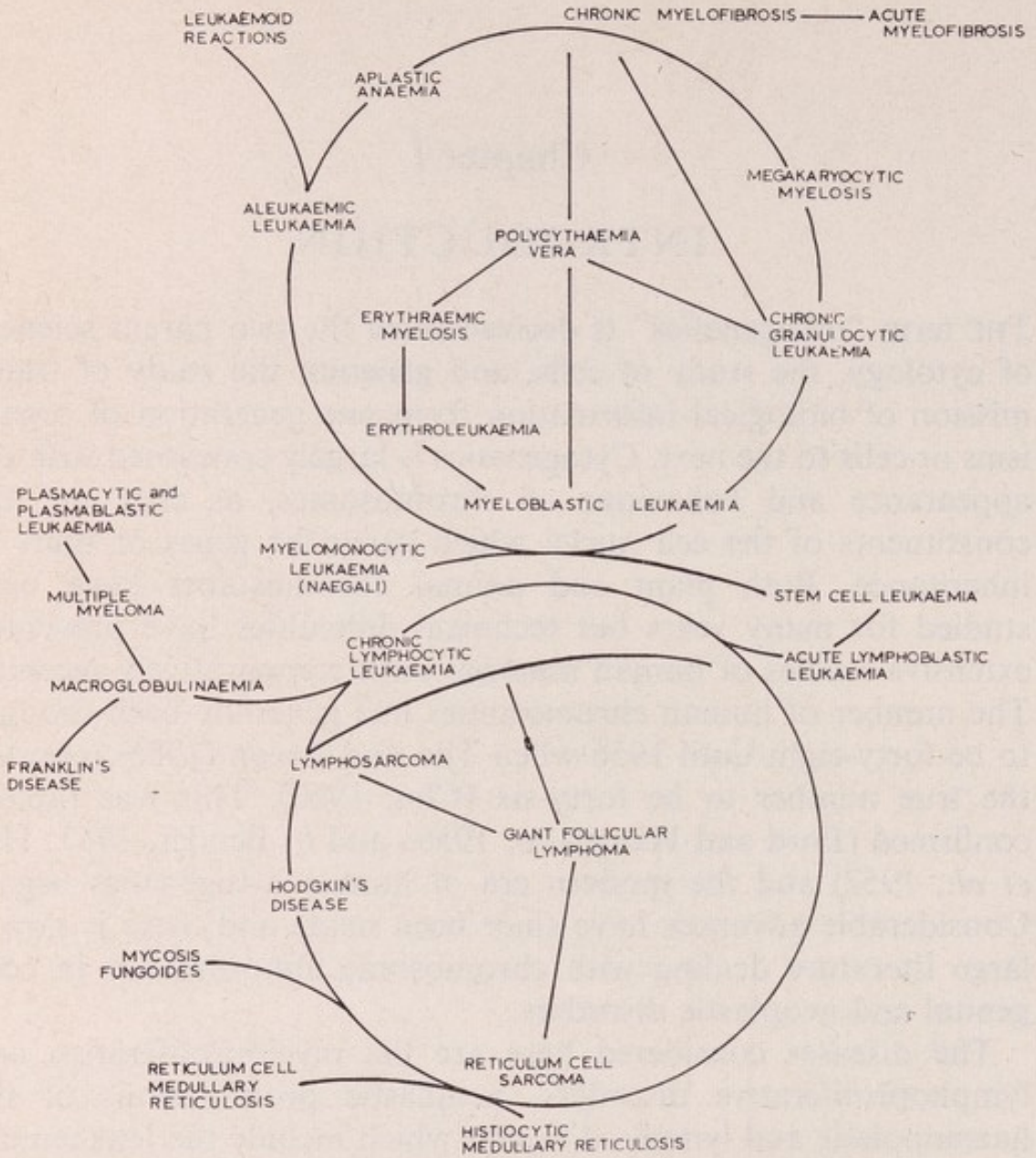

FIG. 1.—The myeloproliferative and lymphoproliferative disorders.

mitoses there are three stages of interphase, a pre-DNA synthesis (G 1) phase, a DNA synthesis (S) phase, and a post-DNA synthesis (G 2) phase. During the S phase, the DNA and nuclear protein of the chromosomes are duplicated.

During mitosis (Figs. 2 and 3), the chromosomes divide and usually each set forms a daughter nucleus; this process, called karyokinesis, is generally accompanied or followed by cytokinesis in which the cytoplasm divides, giving rise to two daughter cells. Mitosis is divided into several phases; during prophase, the chromosomes appear as separate identities, each consisting of two long threads (the chromatids) joined at a centromere. As

prophase progresses, the chromosomes become shorter and more compact and finally the nuclear membrane disrupts. A spindle then forms to which each centromere becomes attached and during metaphase the chromosomes line up on the equatorial plane of the cell. At anaphase the two sister chromatids of each chromosome separate completely to form daughter chromosomes which move

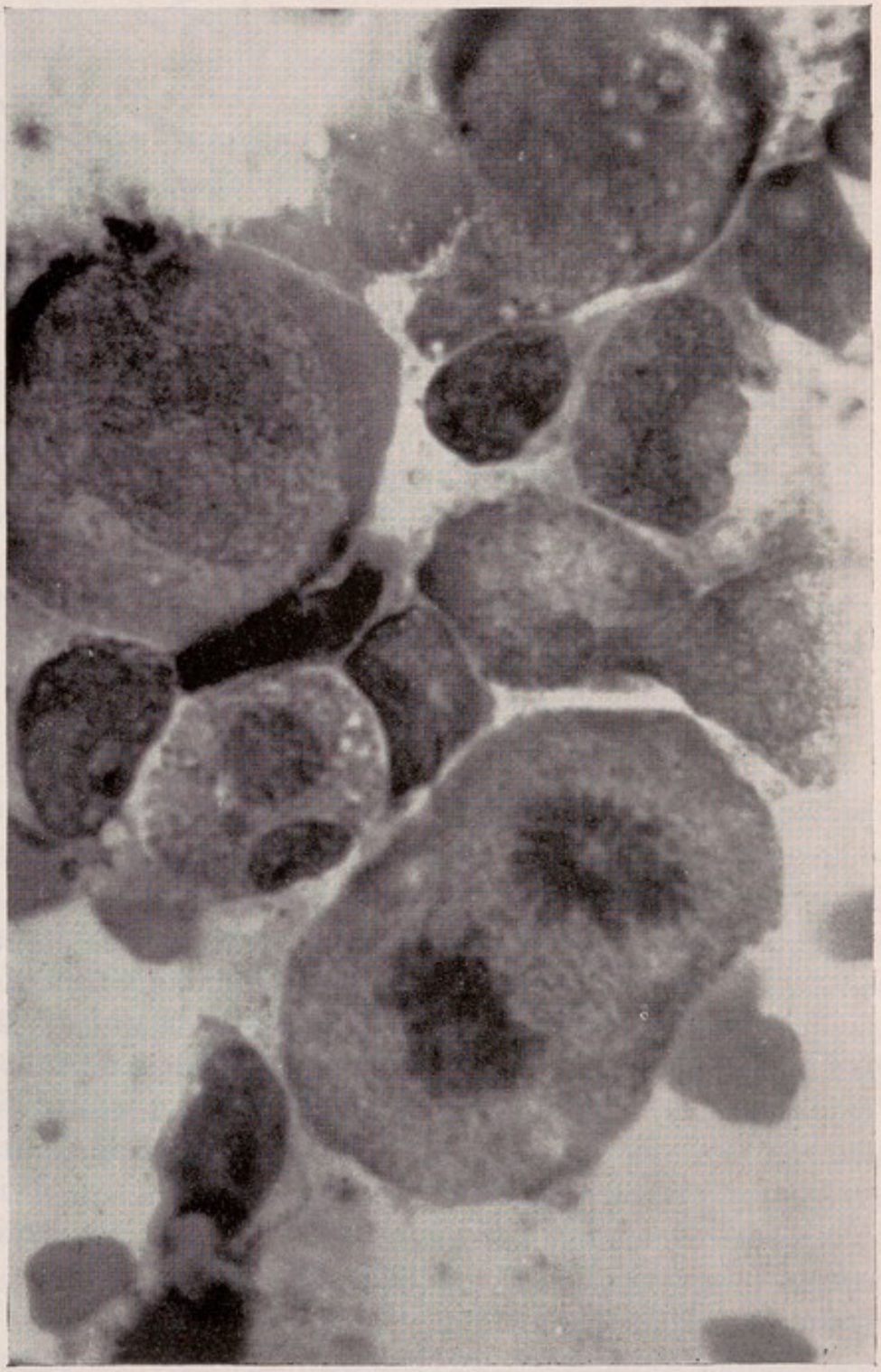

FIG. 2.—Mitotic figure and blastoid cells in a leucocyte culture.

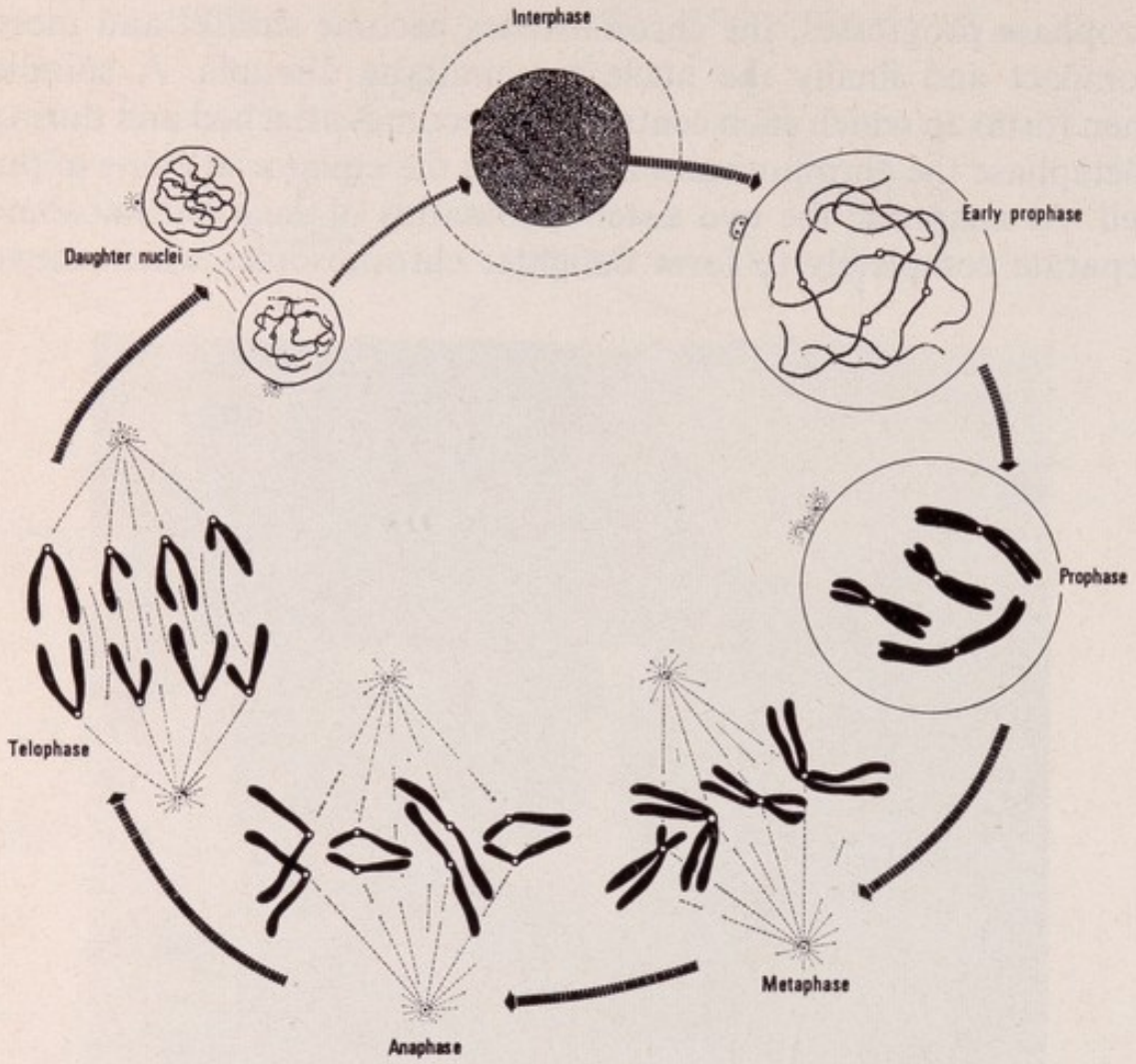

FIG. 3.—Mitotic cycle.

to opposite poles of the spindle. During telophase, each daughter set of chromosomes becomes reconstituted into a nucleus with a membrane.

Meiosis is a special form of nuclear division in which, in two successive phases, four gametes are formed from one somatic cell. A reduction in chromosome numbers occurs so that each gamete contains half the number (haploid) of chromosomes of the somatic cells (diploid). At fertilisation, the fusion of male and female gametes restores the chromosome numbers to diploid. Since this process is not relevant to studies of leukaemic cells, it is not considered further here; those interested will find a description of the process in a textbook of genetics (for example, Berry, 1965; Thompson and Thompson, 1966; Rieger *et al.*, 1968).

Chromosome studies are carried out on material containing cells in mitosis, because it is in metaphase that morphology is

most distinct; these may be obtained either directly from the body or indirectly by culturing cells *in vitro*. The study of direct material is preferable although it has the disadvantage that relatively few dividing cells may be obtained and the recent improvements in techniques were first obtained by culturing cells *in vitro*. Chromosome studies of cultured cells began in 1928 (Kemp) but significant advances did not take place until modern techniques were developed. These consisted of adding colchicine to "stop" mitosis in metaphase, the use of hypotonic solutions to cause the cells to spread and the squashing or air-drying of the metaphases on glass slides to cause spreading of the separated chromosomes (Hughes, 1952; Makino and Nishimura, 1952; Hsu and Pomerat, 1953; Ford and Hamerton, 1956*c*; Tjio and Levan, 1956). Tjio and Levan's important finding on chromosome numbers was made on cells from foetal tissue cultures and in 1958 bone marrow cultures were first used for such studies by Ford and his co-workers. Another commonly used tissue is skin (Harnden, 1960; Hirschhorn and Cooper, 1961), but this is of value in studies in haemopoietic cells only in the demonstration of differences between cells from the two different tissues.

A significant advance was the use of peripheral blood leucocyte cultures. Although the use of such cultures had been suggested in 1932 (Haldane quoted by Chrustchoff, 1935) and metaphases from such cells published in 1935 (Chrustchoff) little progress was made until 1960. This was because blood cultures usually contain few if any cells capable of undergoing mitosis (Chrustchoff, 1935; Bond *et al.*, 1959; Alexander and Spriggs, 1960; Woodliff, 1962). It was then discovered that phytohaemagglutinin induced mitosis in cultures of normal leucocytes. Whilst studying cultures of leukaemic cells, using the gradient technique of Osgood and Krippaehne (1955) in which leucocytes were separated from the blood after precipitation of the erythrocytes by PHA, Nowell noticed that mitoses were present in some of his control cultures of normal leucocytes. He investigated the circumstances favourable to mitosis and found that moderate variations of temperature, pH, oxygen tension, carbon dioxide tension, plasma and cell concentration, as well as the amount of agitation, had little effect. Mitoses were found in all cultures which contained PHA but in none when it was excluded. Nowell concluded that the PHA was an initiator of mitosis in cultures of normal human leucocytes. This finding was soon confirmed and has been used extensively

by cytogeneticists in the study of human chromosomes (Moorhead *et al.*, 1960).

Since artefacts might be produced by *in vitro* culture, direct studies of haemopoietic cells are preferred whenever possible. This is the method of choice for leukaemic bone marrow but cells from blood, lymph nodes and spleen may require to be cultured in order to provide sufficient metaphases for analysis.

Chapter II

METHODS

Introduction

Biological material containing cells which are dividing *in vivo* or which will divide in subsequent *in vitro* culture requires to be specially treated for chromosome studies. The steps may include the following procedures: the use of a spindle poison, such as colchicine, to arrest mitosis at the metaphase stage, hypotonic treatment to swell the cells and cause discrete separation of the chromosomes, fixation, spreading or squashing onto a glass slide, and staining. The chromosomes are then examined microscopically and often photomicrographed. These procedures are considered in this chapter together with a short account of some special methods which are used mainly in research.

Direct Preparations

Introduction

Direct preparations are preferred when there are sufficient dividing cells present in the tissue at the time of collection as this avoids any artefacts due to *in vitro* culture.

Peripheral blood usually contains very few or no dividing cells; however, in some cases of leukaemia or allied disorder, circulating malignant cells may be present and an occasional mitotic figure found; a technique similar to that used for bone marrow (see below) can then be tried.

Lymph nodes may yield a few dividing cells suitable for study but more can usually be found following culture. The nodes are dissected and a cell suspension made which is then treated as for bone marrow. *Splenic tissue* can be dealt with similarly (Yam *et al.*, 1968) but culture is usually required to produce sufficient mitoses for analysis.

Body fluids containing suspended cells are occasionally suitable for processing by a direct method but *skin* and other *solid tissues* are not.

Bone Marrow

Bone marrow may be prepared directly since it usually contains dividing cells. Some workers have pre-treated their patients with a spindle poison prior to aspiration in order to obtain a greater number of metaphases (Bottura and Ferrari, 1960, 1961; Meighan and Stich, 1961*a* and *b*; Traczyk, 1963). Usually, the "colchicine" derivative, desacetylmethyl-colchicine (demecolcine, Colcemid) is injected intravenously in a dose of 0·05 to 0·1 mg per kg of body weight and the bone marrow aspirated 1 to 24 hours later. The ethics of the procedure have been questioned (Stewart, 1960) and such pre-treatment should only be given to patients with established malignant disease. Since chromosomal abnormalities may be produced by such drugs (Hansteen, 1969), their routine use *in vivo* is not recommended. Most workers use a spindle poison *in vitro* but some prefer to omit this stage altogether (Sandberg *et al.*, 1960).

Each laboratory develops its own favoured method of dealing with bone marrow specimens, generally based on the techniques described by Sandberg *et al.* (1961) and by Tjio and Whang (1962, 1965). After hypotonic treatment, fixation and staining, some workers use a squash technique (Makino and Nishimura, 1952); although such preparations can be made permanent this is relatively difficult (Schultz *et al.*, 1949) and air-dried preparations are usually preferred (Rothfels and Siminovitch, 1958; Tjio and Whang, 1965). Spreading may be enhanced by "flaming" (Saksela and Moorhead, 1962; Moorhead, 1964).

Two satisfactory methods are given here:

(1) Marrow is aspirated by a conventional technique and a portion of the specimen (often all that can be spared since some of the aspirate is usually needed for diagnostic purposes) added to 2 ml of a magnesium and calcium free phosphate buffered saline. The specimen is usually processed immediately but if this is impractical it is stored overnight at 4°C. If many large fragments of marrow are present an aliquot is transferred to a further tube of collection fluid and the specimen broken up by pumping with a Pasteur pipette to form a suspension of separated cells. 0·1 ml (10 micrograms) of a demecolcine solution (1 mg in 10 ml of distilled water) is then added to the cells and incubated for one hour at 37°C. In preparations with a high mitotic index this stage may be omitted and still yield suitable metaphases. The specimen is then transferred to a graduated centrifuge tube and spun at low speed (800

rpm) for five minutes when the supernatant fluid is removed and replaced by Hank's solution. After further centrifugation, supernatant is removed and the button of cells re-suspended in 5 ml of 0·075 M potassium chloride solution and incubated at 37°C for five minutes. All but 2 ml of the supernatant is removed, freshly mixed fixative (methanol 3 volumes: glacial acetic acid 1 volume) is layered onto the fluid and the underlying solution gently removed with a Pasteur pipette so that the fixative bathes the button of cells. The deposit is then vigorously pipetted to obtain a uniform cell suspension and after centrifugation and two washings in fresh fixative, they are finally suspended in about 0·5 ml of fixative depending on their concentration. Two or three drops of the suspension are pipetted onto a cleaned wet slide, which is ignited by passing through a flame and, after burning, any excess fluid is wiped or flicked off the slide. The preparation should be checked microscopically and if necessary the cells concentrated by centrifugation and re-suspension in a lesser amount of fixative, or diluted by adding a greater volume. Suitable preparations are stained as described on p. 14.

(2) Marrow aspirate is added directly to 20 ml of cold Earle's solution and rocked to suspend the cells; 7 ml of the suspension is then added to 28 ml of 0·44 per cent sodium citrate solution and mixed by repeated inversion. This hypotonic treatment is allowed to continue at room temperature for 15 minutes, after which the preparation is centrifuged lightly and the supernatant removed; 5 ml of fixative (methanol acetic) is added and mixed with the cells and the suspension is then treated as described for Method 1.

Culture Methods

Peripheral Blood

Mention has already been made of the circumstances leading to the widespread use of peripheral blood cultures for chromosome studies (p. 5) and the many methods in use today are based on that described by Moorhead *et al.* in 1960 (Moorhead, 1964; Mellman, 1965). Mitotic figures are obtained from normal blood cells by culturing them with PHA or some other mitogenic agent which induces some of the lymphocytes to divide (leukaemic cells may mitose without stimulation). Whole blood can be used and micro-techniques based on adding a few drops to a culture medium have been described (Arakaki and Sparkes, 1963; Tips *et al.*,

1963); however, in most methods the leucocytes are first separated from the red cells and although this can be applied to small quantities of blood (Edwards, 1962; Froland, 1962) most techniques use from 2 to 10 ml. The blood is usually collected into heparin but citrate anticoagulant can also be used. Leucocyte-rich plasma can be obtained by allowing the red cells to settle by gravity or centrifugation alone or following the addition of fibrinogen, dextran or PHA (Skoog and Beck, 1956). Some workers like to remove granulocytes by magnetism following their ingestion of iron particles; other methods include filtration, differential absorption and density gradient techniques (Hastings *et al.*, 1961; Carstairs, 1962; Cooper and Hirschhorn, 1962; Speed and Lawler, 1964; Yam *et al.*, 1968; Pentycross, 1968; Boyle and Chow, 1969). Cultures are generally set up as soon as practicable but separated leucocytes in their own plasma may be stored at 5°C for up to four days (Mellman *et al.*, 1962) and mitoses may even be obtained from whole blood stored for up to 22 days at 4°C (Petrakis and Politis, 1962). Preservation of leucocytes in a special medium and storage at −180°C is also possible (Ducos and Colombies, 1968).

A variety of media have been used, most containing about 20 per cent of a protein solution such as autologous plasma, human AB serum or foetal calf serum and 80 per cent of an amino-acid and dextrose containing salt solution, such as Medium 199 or Eagle's medium (Woodliff, 1964).

Small screw-cap containers are usually used as culture vessels and leucocyte suspensions cultured at 37°C. Many media contain a phenol red pH indicator and a carbonate dependent buffer; the pH of these can be adjusted by gassing with 5 per cent CO_2 in air (to make it more acid) or by loosening the cap to allow CO_2 to "blow off" to make it more alkaline. Humidified incubators with an adjustable gas phase are useful especially when culturing cells in open petri dishes but are not necessary for routine work.

PHA is obtained commercially (Difco M) as a sterile powder which is reconstituted with distilled water. Each new preparation should be tested for potency by comparing it with a previous batch known to be mitogenic. Some workers prepare their own material but those commercially available are now so satisfactory that this is hardly worthwhile.

The following method has given consistent and satisfactory results (Woodliff, 1962).

Method.—Blood from patients with a high erythrocyte sedimen-

tation rate (common in leukaemia) is collected into a sterile syringe from a cubital vein and 10 ml placed in a sterile universal bottle containing 1 mg of heparin dissolved in 0·1 ml of distilled water. Blood from patients without a high ESR is collected similarly and added to a bottle containing 0·5 ml of sterile dextran solution (Dextraven—6 per cent dextran in saline) in addition to the heparin. After mixing, the specimens are allowed to stand at room temperature for half an hour to two hours, the leucocyte rich supernatant plasma is removed and a cell count performed; the remainder of the specimen is centrifuged to produce cell free plasma. Where necessary, the cell count is adjusted to 5–10,000 leucocytes/μl by the addition of patients' cell free plasma or, if unavailable, with sterile AB plasma or serum. The cell suspension is then diluted with four volumes of tissue culture fluid 199 (Difco M) or Eagle's basal medium, and 0·1 ml of a PHA M (Difco) solution, prepared according to the direction of the manufacturers, is added to each 5 ml of culture fluid if mitotic stimulation is required. Depending upon the material available and the purpose of the culture, a number of aliquots are put up in 2 to 10 ml amounts in 15 ml screw-capped bottles and when necessary the pH is adjusted to between 7·0 and 7·2 by the addition of 5 per cent carbon dioxide in air. Cultures are incubated at 37°C and harvested at approximately 24, 48 and 72 hours after prior treatment of two to four hours with demecolcine to a final concentration of 2 μg/ml. On harvesting, cell clumps are broken up by pipetting and the culture transferred to a suitable tube for centrifuging for five to ten minutes; the supernatant is then discarded and the cells washed with Hank's solution. After again centrifuging, the button of cells is suspended in 2 ml of Hank's solution which is then diluted with 6 ml of pre-warmed 37°C distilled water. After thoroughly mixing, the cells are incubated for a further five minutes after which they are centrifuged at low speed for 10 minutes and all but 2 ml of the supernatant removed. 3 ml of freshly made fixative (3 volumes methanol, 1 volume glacial acetic acid) are layered on top of the aqueous phase and this is gently removed from beneath the fixative with a Pasteur pipette so that the methanol and glacial acetic acid bathe the button of cells at the bottom of the tube. The cell button is then broken up to make a uniform suspension by gently pumping with a pipette, and the cells are again centrifuged, washed with fresh fixative twice and finally suspended in about 0·5 ml of fresh fixative. Three drops of the suspension are dropped onto a water

wetted slide and allowed to spread over the surface, the suspension is ignited and after the flame has burnt out any surplus drops are removed by flicking; the drying process is completed by suspension high above a bunsen flame. One slide is examined microscopically for the right density of cells and if suitable other slides are fixed and stained. If necessary, the original suspension can be concentrated or diluted to produce a more suitable cell density.

Bone Marrow

Early studies of bone marrow chromosomes were made after 5 to 15 hours of culture (Ford *et al.*, 1958; Sandberg *et al.*, 1960); subsequently, several culture techniques were tried (Woodliff, 1964) but generally abandoned in favour of a direct method. However, for special purposes, for example to obtain bone marrow fibroblasts, cultures can be set up and processed in a similar way to that described below for skin cultures.

Lymph Node

Diagnostic lymph node biopsies can be cultured for chromosome studies; a variety of techniques have been described but generally tissues are bathed in culture medium, minced with fine scissors and then pipetted to break up the fragments into a cell suspension. The concentration is then adjusted to about 700 to 15,000/μl in a tissue culture fluid consisting of 20 to 30 per cent serum or plasma and 70 to 80 per cent of medium 199 (Baker and Atkin, 1965; Spiers and Baikie, 1966 and 1968*a*; Kajii *et al.*, 1968). Aliquots are incubated for up to 48 hours and then treated as for blood cultures; a brief culture increases the number of mitoses without the possible selective growth of a particular karyotype (Spiers and Baikie, 1968*a*). Long term cultures generally lead to changes such as an increase in aneuploidy (Trujillo *et al.*, 1967).

Spleen

Splenic cell suspensions obtained either by aspiration *in vivo* or from surgical or post-mortem specimens can be cultured by one of the methods used for blood or other tissues (Spiers and Baikie, 1965*b*; Pawelski *et al.*, 1967). Short term cultures without PHA and longer term cultures with PHA may be set up depending upon the purpose of the analysis.

Skin and Other Tissues

Cell suspensions cannot be readily obtained from skin and this applies also to many other tissues; it is therefore necessary to culture them *in vitro* and harvest the cells which grow from the explant for chromosome analysis.

A variety of techniques have been described (Edwards, 1960; Harnden, 1960; Hsu and Kellog, 1960; Froland, 1961). After cleansing the skin with ether, a small portion ($1 \times 1 \times 5$ mm) is removed by means of a scalpel or razor blade. Specimens should be processed as soon as possible after collection but successful culture can be made after storage in culture medium for several days. The specimen is cut into half millimetre squares and cultured in flasks, being held against the glass well either by a plasma clot, cellophane or a glass cover slip (Woodliff, 1964). When sufficient cells have grown from the explant, they are harvested by trypsinization using a calcium and magnesium free buffered saline solution and re-cultured in further flasks. When sufficient growth has occurred the cultures are treated with a spindle poison, released from the glass by trypsin, treated hypotonically and processed as for blood cells (see above).

Examination of Metaphases

Both direct and culture methods yield fixed metaphases on glass slides; to analyse these it is usual to stain them, examine them microscopically and, if a full analysis is required, to photograph them and prepare a karyotype from the print.

Staining

Unstained metaphases can be examined by using phase-contrast or low illumination microscopy and this enables the chromosomes to be counted and drawn. This has the advantage of speed but most workers prefer to stain the chromosomes and make permanent preparations which are more convenient for photography and reference purposes, as they can be kept for a number of years without deterioration. Several staining methods have been used; in some, the cells are stained prior to preparation of the slides. However, in most the slides containing the fixed cells are either bathed or immersed in stain. A variety of stains can be used, including carbol fuchsin, Giemsa, orcein, Schiff's reagent (Feulgen reaction) and gallocyaninchrome alum (Rothfels and Siminovitch,

1958; Lima-de-Faria, 1961; Tips *et al.*, 1963; Mendelsohn *et al.*, 1966). The May-Grunwald Giemsa method is recommended; the slides are immersed in May-Grunwald (MG) stain (20 per cent), washed in buffered distilled water and then immersed in Giemsa stain (30 per cent) in buffered distilled water for a further 10 minutes. After washing in buffered distilled water they are air dried and mounted in DPX. The May-Grunwald stain is made by dissolving 0·3 g in 100 ml of methanol after which the solution is placed in a water bath at 60°C for one hour and then allowed to cool; it is shaken at intervals, allowed to stand for 24 hours, and then filtered for use. The Giemsa solution is made by dissolving 1 g of stain (Hopkins and Williams) in 66 ml of glycerol and heating at 56°C for 90 minutes. Sixty ml of methanol is added, the mixture allowed to stand for several days at room temperature and then filtered. Phosphate buffer consists of 50 ml of 0·2 M potassium dihydrogen phosphate and 23·7 ml of 0·2 M sodium hydroxide. This solution should have a pH of 6·8 and 2 ml are added to 100 ml of water for the working solution.

Microscopy

A good light microscope with high resolution oil-immersion optics is needed for chromosome studies. Care must be taken in the selection of the instrument, taking into consideration funds available, the reputation of the manufacturers, the suitability for phase, interference and fluorescent microscopy, the nature of the photographic attachments and so on. Those new to microscopy should read one of the standard texts and articles dealing specifically with the examination of chromosomes (Allen, 1951; Barer, 1956; Martin and Johnson, 1958; Needham, 1958; Christensen, 1965; Runge, 1965); if possible, the advice and tuition of someone already experienced in the field should be sought.

The microscope used in the author's laboratory, a Reichert Zetopan, can be recommended, but similar results can be obtained with comparable Leitz and Zeiss models and recently an Olympus has produced satisfactory results.

In addition to the high quality microscope, a simpler less expensive model can be used for a preliminary look at the slide for staining quality. A separate microscope permanently set up for phase contrast microscopy is useful as this gives a clear view of unstained objects and can be used for the examination of living cells. An inverted microscope, such as the Olympus, is convenient

for examining cell cultures but it is not an essential instrument for routine cytogenetics.

Photography

The production of photographs of metaphases adequate for karyotyping requires good equipment, careful attention to detail and experience. Reading helps (Kodak, 1957; Allen, 1958; Christensen, 1965) but a short apprenticeship with a good photomicrographer is invaluable.

Two types of camera are in common use, the 35 mm, using a roll film and producing negatives 24 × 36 mm in size, and larger cameras using roll film or plates producing negatives about 100 × 125 mm. The smaller negative means that prints have to be enlarged and this can result in some loss of definition; however, the convenience of having up to 36 exposures on one film is a compensating advantage. A built-in exposure meter is useful but not essential because test strip films can be made.

For routine use an objective and a matching eyepiece are selected; the magnification is a product of the two lenses and in the case of 35 mm cameras with a short focal length a reduction factor may have to be applied to give the true primary magnification. For example, a magnification of 500 might be derived from a ×100 objective, a ×10 eyepiece and a ×0·5 supplementary camera lens. For the prints, the magnification of the enlarger, for example, ×4 should be added. It should have a first-class lens system and high contrast printing paper is usually used. If necessary the magnification can be checked by photographing a stage micrometer. With large negatives direct contact printing may be satisfactory. The metaphases selected for photography should be spread so that the chromosomes do not overlap but are not too widespread. There should be no dirt, scratches or other artefacts in the field. A filter suitable to the stain used for the chromosomes is placed in the light beam (e.g. a green filter is used for May-Grunwald Giemsa stained chromosomes). Careful attention should be paid to focusing because on direct microscopy one can obtain a third dimensional image whereas only two dimensions will be present on the print. Occasionally, it is useful to take two photographs at slightly different levels.

Karyotype Analysis

The chromosomes on the photographic print are counted and checked against the direct count; they are then cut out with scissors

and arranged in order according to their size and grouping (see Fig. 4 and page 19). When all the chromosome pairs have been arranged to the satisfaction of the analyst they can be permanently mounted on stiff paper using double-sided adhesive tape. For reporting purposes, the print of the metaphase and its karyotype can be re-photographed or, more easily, photocopied.

Special Methods

The methods outlined above can be used routinely; more experimental and time consuming methods are used in research and may occasionally have a clinical application; they include auto-radiography, measurements of chromosomes, automated scanning of metaphases and electron microscopy.

Autoradiography

Autoradiography techniques in which radioactive precursors of DNA, such as tritiated thymidine, are added to cell cultures, have been used to determine the timing and pattern of human chromosomes replication and to help in their identification (Lima-de-Faria *et al.*, 1961; Morishima *et al.*, 1962; Moorhead and Defendi, 1963; Kikuchi and Sandberg, 1964). The application of such techniques in studies of leukaemic cells has been pursued by some in an endeavour to pick up abnormalities which are not visible in standard preparations.

Chromosome Measurements

A variety of methods of measuring chromosomes have been employed; as they are generally time consuming they have been used in research into specific problems rather than having a general application. Shown photographically negatives of the metaphase are projected on to a screen and measurements of chromosome lengths and areas made. By relating various parameters the relative size of the chromosomes can be determined and indices of the relative arm lengths calculated; these may be of value in identification of the individual members of the various groups (Fitzgerald, 1965*a* and *b*; Gilbert, 1966; Giannelli and Howlett, 1966, 1967). Measurement of DNA content can also be made if an integrated spectrophotometer is available.

Automated Scanning of Chromosomes

Survey of a larger number of metaphases is time consuming and an automatic system is required to scan metaphases and to produce karyotypes giving statistics on chromosome lengths, breadths, shapes and areas; several systems are being developed (Gilbert, 1966; Ledley *et al.*, 1966; Ledley and Ruddle, 1966). The FIDAC and BUGSYS system developed by Dr. Ledley and his colleagues at the National Biomedical Research Foundation, Silver Springs, Maryland, makes use of photographic negatives of metaphases; the film is then placed in a flying-pot scanner (FIDAC —Film Input to Digital Automatic Computer) and the information fed into a computer programmed to recognize, count and classify the chromosomes. The time needed is about 20 seconds, compared with an estimated one to three hours for conventional karyotyping. The term BUGSYS is given to the picture processing and measuring system, which used a collection of programmable pointers considered as a family of "bugs" (hence BUGSYS bug system); it is compatible with FORTRAN II language. A disadvantage of this system is that it does not eliminate photography and the equipment is too expensive to be considered for most centres. Possibly a centre to which negatives could be sent would be appropriate for each country. Its advantage is that it has developed to the stage of being useful; work on a similar system is being carried out in London (*British Medical Journal*, 1968). Another system being developed in Philadelphia scans the slides for suitable metaphases and feeds chromosome measurements directly into a computer (Mendelsohn *et al.*, 1966, 1969). This has not yet reached the stage of being commercially available but has considerable potentialities. It is based on optical information obtained from a densitometric measurement by a special purpose cytophotometer linked to a computer (CYDAC).

Electron Microscopy

Electron microscopy of chromosomes is time consuming and costly; however, recent advances in this kind of technique are promising. These methods provide pictures of chromosomes at a greater magnification than can be obtained by a light microscope and can also reveal electron density and profile type pictures (Schultz *et al.*, 1949; Christenhuss *et al.*, 1967; Neurath *et al.*, 1967; Neurath, 1968; Lampert *et al.*, 1969). It is hoped that this

tool will reveal abnormalities in leukaemia that have escaped detection by light microscopy.

Chapter III

NORMAL HUMAN CHROMOSOMES

Introduction

The events leading to the establishment of forty-six as the normal number of human chromosomes were outlined in Chapter 1 and the methods used in their study in Chapter II. Here the findings in cells from normal subjects are considered in more detail.

Human Karyotype

Studies of metaphases from the cells of normal subjects have shown that the 46 chromosomes are made up of 22 autosomal pairs and one pair of sex chromosomes (XX in the female and XY in the male); this number is called *diploid* (2n). In some tissues, such as liver, *polyploid* (Xn) cells containing multiples of the diploid number are found; and the gonads contain *haploid* (n) germ cells with 23 chromosomes.

The term *karyotype* is applied to a systematic arrangement of the chromosomes of a single cell, as illustrated in the lower part of Fig. 4; it is also used to mean the typical findings in an individual species. The term idiogram is reserved for a diagrammatic representation of the karyotype which may be based on the measurements of the chromosomes in several or many cells (Fig. 5).

The autosomes have been divided into seven groups, A to G and numbered according to their length and position of the centromere (Book *et al.*, 1960; Denver Conference, 1960; *Lancet*, 1960, 1961; Patau, 1960, 1961*a* and 1965; London Conference, 1963; Chicago Conference, 1966; Table I).

The centromere, a non-staining area joining the two chromatids, may be median, submedian, subterminal or terminal (Fig. 6, Levan *et al.*, 1964*a*). The median position varies from true median; in which case the chromosome arms are of equal length, to a position five-eighths along the chromosome; submedian from there to three-quarters; subterminal from there to seven-eighths, and terminal from there to the end. Telocentric chromosomes are

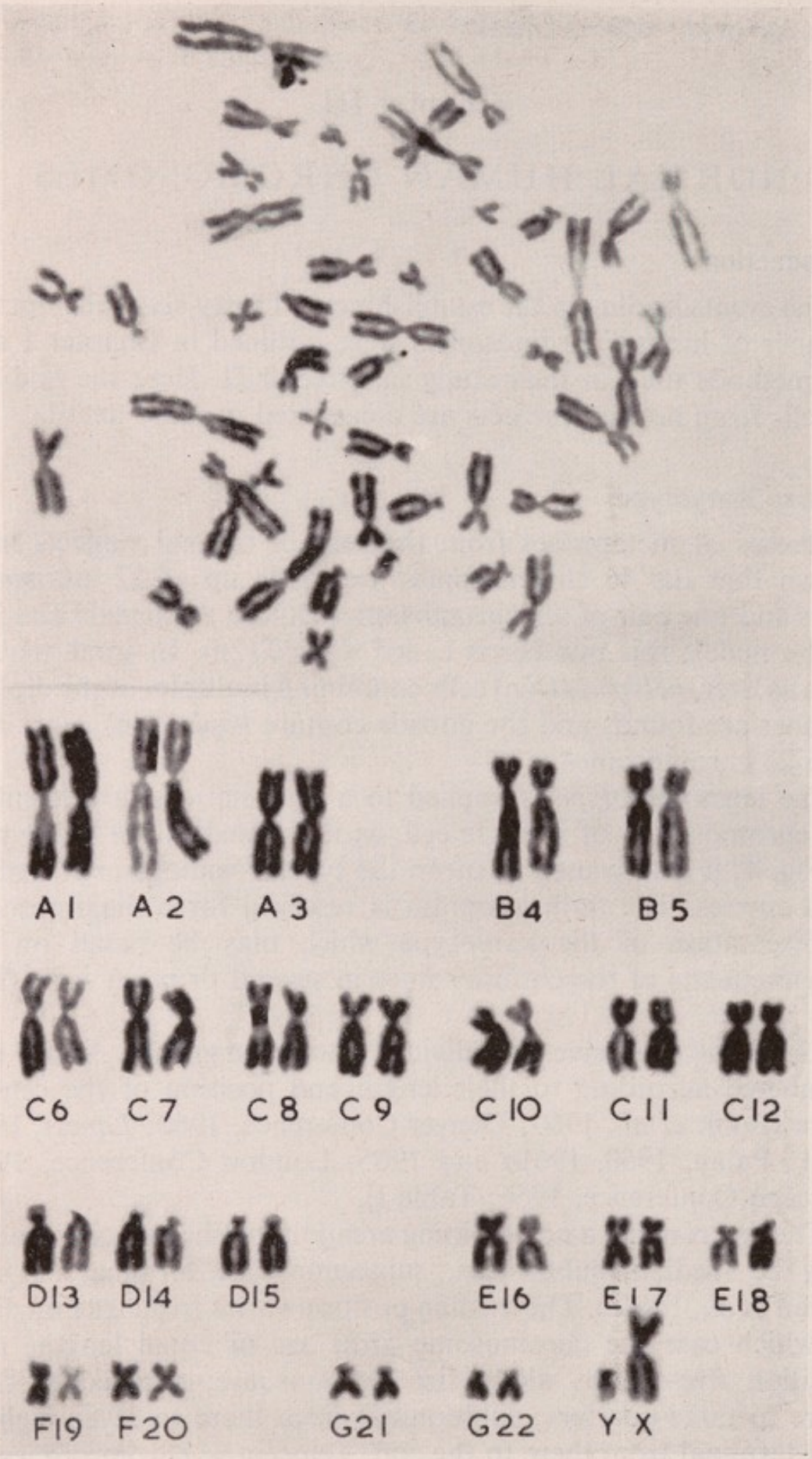

FIG. 4.—Normal human male metaphase and karyotype 46 XY from a phytohaemagglutinin peripheral blood culture (FF300328M).

TABLE I

NOMENCLATURE SYMBOLS

A–G	the chromosome groups
1–22	the autosome numbers (Denver System)
X, Y	the sex chromosomes
diagonal (/)	separates cell lines in describing mosaicism
plus sign (+) or minus sign (−)	when placed immediately after the autosome number or group letter designation indicates that the particular chromosome is extra or missing; when placed immediately after the arm or structural designation indicates that the particular arm or structure is larger or smaller than normal
question mark (?)	indicates questionable identification of chromosome or chromosome structure
asterisk (*)	designates a chromosome or chromosome structure explained in text or footnote
ace	acentric
cen	centromere
dic	dicentric
end	endoreduplication
h	secondary constriction or negatively staining region
i	isochromosome
inv	inversion
inv(p+q−) or inv(p−q+)	pericentric inversion
mar	marker chromosome
mat	maternal origin
p	short arm of chromosome
pat	paternal origin
q	long arm of chromosome
r	ring chromosome
s	satellite
t	translocation
tri	tricentric
repeated symbols	duplication of chromosome structure

those with the centromere at the very end and are not found in cells from normal humans. The terms metacentric and acrocentric are frequently used in the literature somewhat loosely; here metacentric is used for those chromosomes in which the centromere is median and submedian, that is up to three-quarters of the way

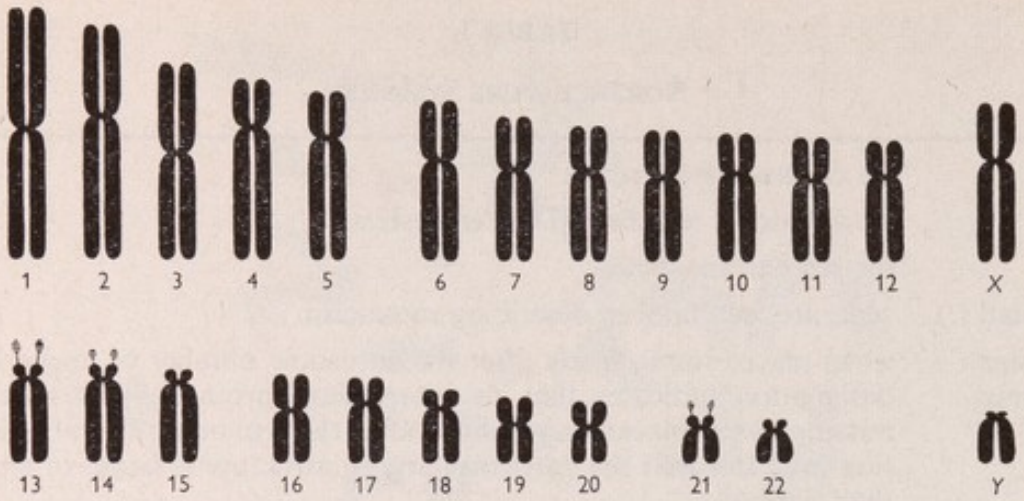

FIG. 5.—Normal human idiogram, based on the Standard System of Nomenclature of Human Mitotic Chromosomes (Proposed by the Denver Conference).

along the length of the chromosome and acrocentric for those in which the centromere is subterminal to terminal, that is more than three-quarters of the way along the chromosome.

In the first group (A), there are three pairs of easily recognized large chromosomes with median centromeres (1, 2 and 3).

In the second group (B), there are two large pairs (4 and 5) with submedian centromeres.

In the third group (C), there are seven pairs (6 to 12), with submedianc entromeres and the X chromosome is morphologic-

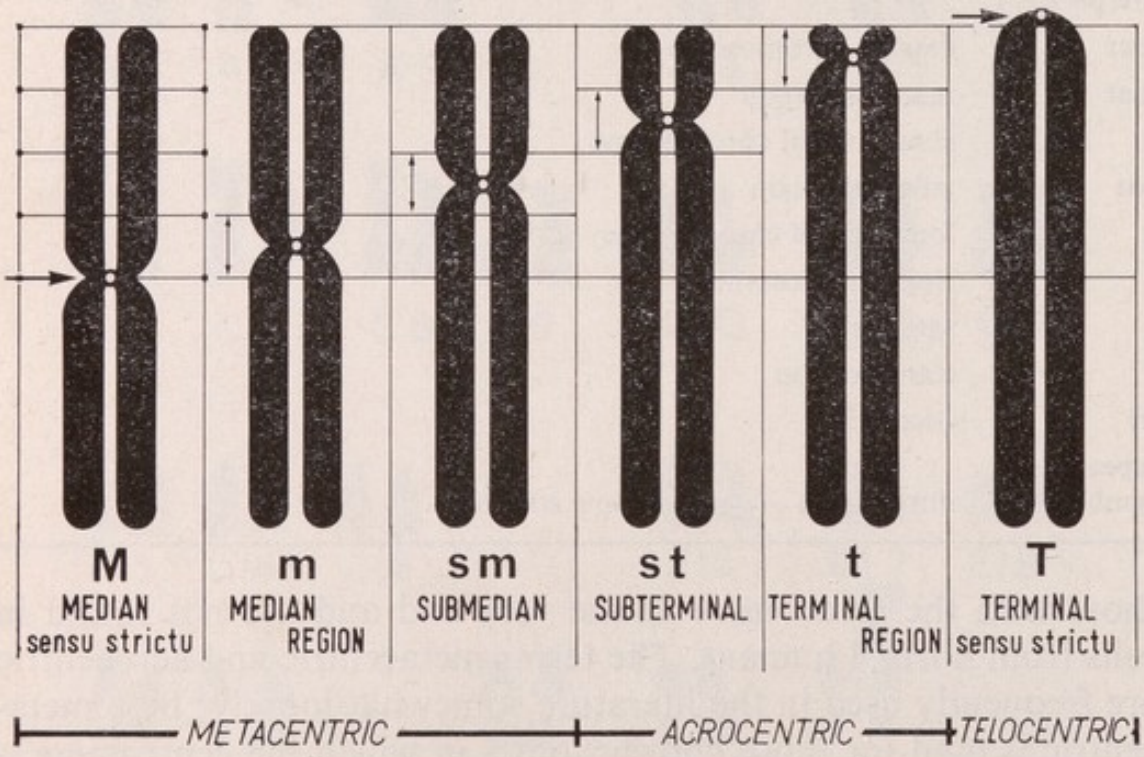

FIG. 6.—Centromere positions and nomenclature.

ally similar, so that in this group (C–X), there are 14 autosomes and one X chromosome in males and 14 autosomes and two X chromosomes in females.

In the fourth group (D), there are three pairs (13, 14 and 15). These are medium-sized chromosomes with subterminal centromeres and are often known as the large acrocentrics.

In the fifth group (E), there are three pairs (16, 17 and 18) of small to medium-sized chromosomes, with a median (16) or submedian (17) centromere.

In the sixth group (F), there are two pairs (19 and 20) of small chromosomes with a median centromere.

In the seventh group (G), there are two pairs (21 and 22). They are the smallest of the chromosomes, have subterminal centromeres and divergent long arms; they, together with the Y chromosome, are often called the small acrocentrics.

The Y chromosome is similar to these, having a subterminal centromere; it varies somewhat in size and shape and usually the long arms are parallel to one another rather than divergent. Sometimes it may be difficult to distinguish the Y from a G chromosome and so they are often grouped together as G–Y.

Small spherical bodies lying a short distance from the end of the short arms of acrocentric chromosomes are called satellites. They are joined to the rest of the short arm by a narrow thread and are illustrated diagrammatically in Fig. 5 on chromosomes 13, 14 and 21. Satellites have also been found on other acrocentric autosomes but not on the Y chromosome.

Secondary constrictions are sometimes seen as non-staining areas on a chromatid or chromosome; they bear some resemblance to the centromere or primary constriction but are not a constant feature. They are seen more often on some chromosomes (Nos. 1, 9 and 16) than on others (Ferguson-Smith and Handmaker, 1961, and 1963; Saksela and Moorhead, 1962; Sasaki and Makino, 1963).

Whilst normal human chromosomes can be divided into the seven groups, A, B, C–X, D, E, F and G–Y, it is often not possible to indicate with certainty the individual chromosomes and comparatively few (such as 1–3, 16 and Y) can be identified on morphological grounds alone. Chromosome measurements and autoradiography may further help in identification (4 and 5, the D group, 17 and 18), but it is still not possible to distinguish between 19 and 20. Members of the C–X group of chromosomes

cannot usually be distinguished from one another, except for one pair often showing secondary constriction, usually called C′ or C9 and in females one of the Xs which is late replicating (Patau, 1961*a*).

Normal Variations

The term normal is used variously in medicine to include the ideal, the most common or that not associated with any disease state. In chromosomes studies, a certain amount of variation is found in controls taken from "normal" subjects and this must be defined so that the pathological state may be more clearly recognized.

Numerical deviations from the normal haploid, diploid or polyploid number of chromosomes is called aneuploidy; it is influenced by techniques and experience and may be artificial, produced by the loss or addition of chromosomes to metaphases during preparation, or be real occurring *in vivo*. In our laboratory, control blood cultures were collected from associates and friends in apparently normal health, who were not, however, medically examined. Since bone marrow aspiration usually causes some discomfort volunteers are not so readily obtained. Control subjects have included patients with normal blood and bone marrow findings, together with a few normal subjects who volunteered for the examination.

Aneuploidy in blood cultures is usually about 5 per cent (range 0–18 per cent) and in bone marrow about 10 per cent (range 0–20 per cent).

In early studies, up to 36 per cent of cells from normal peripheral blood cultures were reported as aneuploid (Nowell and Hungerford, 1960*a*). By 1962, however, Jacobs had found that not more than 6 per cent of cells were aneuploid and of these more were hypodiploid (5 per cent) than hyperdiploid (1 per cent). Similar findings are general (e.g. Nasjleti and Spencer, 1968), and a figure of 5 per cent can be taken as an arbitrary upper limit of "normal".

A statistical study of a larger number of metaphases from healthy volunteers, hospital inpatients and relatives of patients by the Edinburgh group has shown that aneuploidy increases with age and that there is a sex difference (Jacobs *et al.*, 1961; Buckton *et al.*, 1962*a*; Jacobs *et al.*, 1963*a*; Court Brown, 1967). These findings have generally been confirmed in another population

by Hamerton *et al.* (1965). Aneuploidy in the female was around 4–6 per cent up to the age of 54; thereafter it was more than 10 per cent. Analysis showed that in the hypodiploid metaphases some chromosomes are more frequently absent than in others and in females the most common hypodiploid line was 45 C–; it was concluded that the missing chromosome was an X. The age change in males was less marked but there was evidence of an increasing loss of one of the G–Y chromosomes and this was more often identified as a Y. Sandberg *et al.* (1967*a*) using blood samples from an unselected population confirmed that hypodiploidy was increased in females but only above 65 years of age; they found no significant hypodiploidy in males.

Occasional polyploid metaphases (0–5 per cent) may also be found in cells from normal cultures and chromatid gaps and breaks in up to 4 per cent (Nasjleti and Spencer, 1968).

Bone marrow metaphases generally show a greater degree of aneuploidy than those prepared from peripheral blood cultures; possibly because they are more difficult to prepare there are more artefacts (Ford, 1959). Short-term cultures of bone marrow from 10 normal subjects contained about 15 per cent aneuploid metaphases but less than 3 per cent were hyperdiploid (Court Brown *et al.*, 1960). Sandberg *et al.* (1960) using a direct method studied cells from 10 subjects and found on average that 8 per cent of the cells were hypodiploid and 2 per cent hyperdiploid, later (Sandberg *et al.*, 1961) a figure of $12{\cdot}2 \pm 0{\cdot}8$ per cent was given. In our laboratory, aneuploidy ranges from 0 to 20 per cent were obtained with a mean of 9·6 per cent. Our arbitrary normal is up to 10 per cent and we are suspicious if 11 per cent are more aneuploid, especially if more than 3 per cent are hyperdiploid (Onesti and Woodliff, 1968).

Morphological variations may occur in up to 3 per cent of adults. Common changes are lengthening of the short arm of an acrocentric chromosome in groups D or G, and variation in the size of the Y chromosome. A smaller number (0·5 per cent) of structural changes in the autosomes may be found in normal adults (Court Brown, 1967).

Chapter IV

CHROMOSOMAL ABNORMALITIES

Introduction

The preceding chapter dealt with the normal human karyotype and the variations which could occur in the normal population: this chapter is concerned with the abnormalities that might be expected in disease or following exposure to various agents either *in vitro* or *in vivo*. For convenience, they are divided into numerical and morphological variations, although both are often present in the same cell or in different cells from the same organism. Sometimes more than one cell line with one or more abnormal clones or a mixture of abnormal clones may be present in the same material; this is called mosaicism.

Numerical Variations

Deviations from the normal haploid, diploid or polyploid number of chromosomes is called aneuploidy and occurs to a limited extent in cells from normal subjects. Five per cent aneuploidy in the peripheral blood and 10 per cent in the bone marrow can be taken as the upper limit of normal although a greater aneuploidy may be found in cells from some apparently normal subjects. Both hypodiploidy (less than 2n chromosomes) and hyperdiploidy (more than 2n chromosomes) may be produced by uneven division; if during metaphase more chromosomes (say 47) go into one daughter cell and less (say 45) into another, the progeny of such cells, if viable, will be aneuploid. This may apply to both meiotic and mitotic divisions and is called non-disjunction; it is a failure of the two members of a chromosome pair to separate at metaphase. A similar state of affairs occurs if chromosomes do not line up properly during the spindle phase or fail to move at anaphase with the result that they may be included in the wrong daughter cell or not included in either. Increased aneuploidy is found in a variety of diseases or following various treatments; it may be due to random drop out or increase of

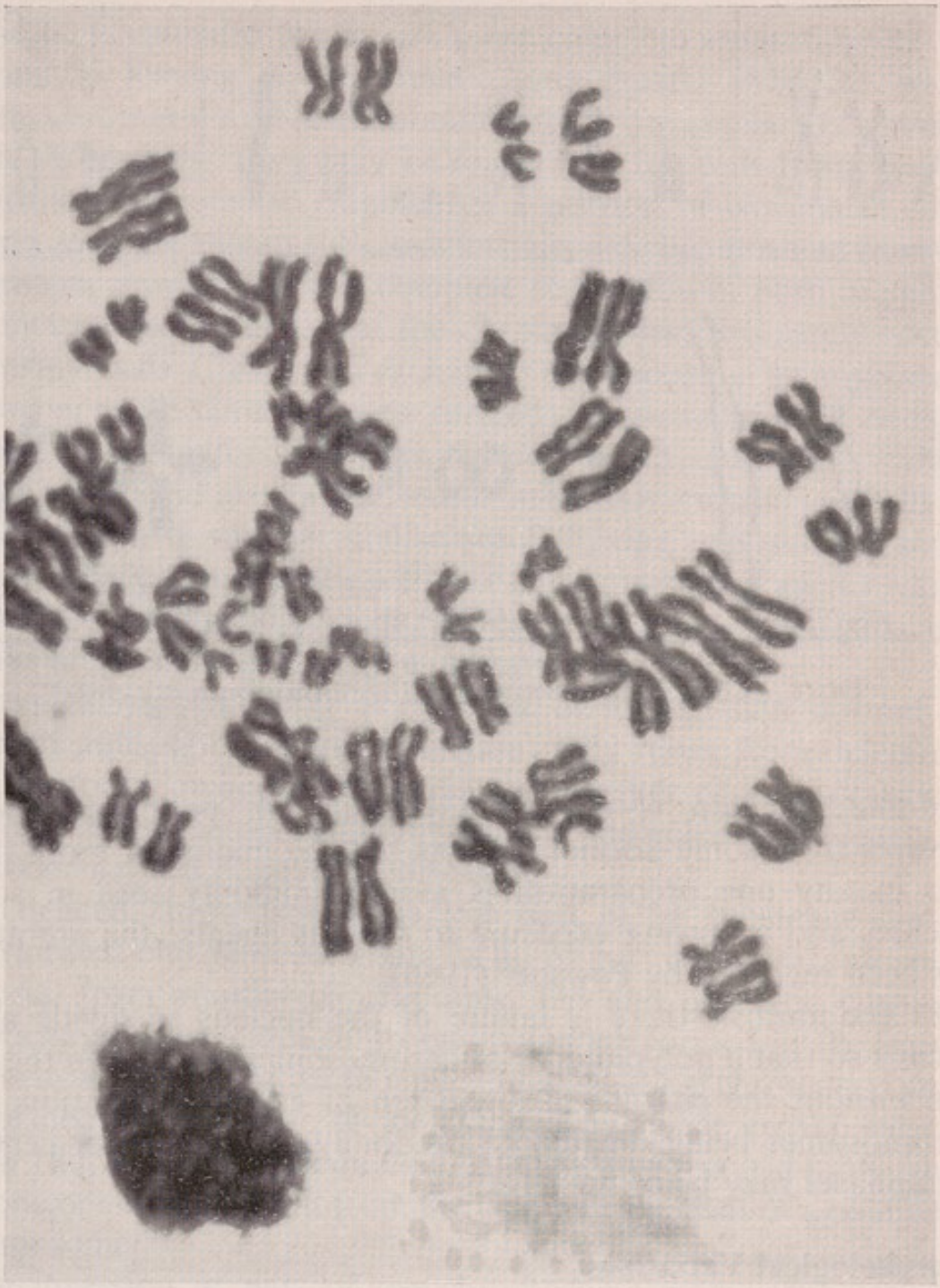

FIG. 7.—Endoreduplication in a cell from a three-day phytohaemagglutinin peripheral blood culture, from a patient with chronic granulocytic leukaemia (SM269611. 0033F).

various chromosomes or constitute an abnormal cell line with a constant change.

In endoreduplication the chromosomes replicate during interphase to form diplochromosomes; twice the number of chromosomes appear at the next metaphase and the daughter chromosomes are closely associated, giving a characteristic appearance (Fig. 7).

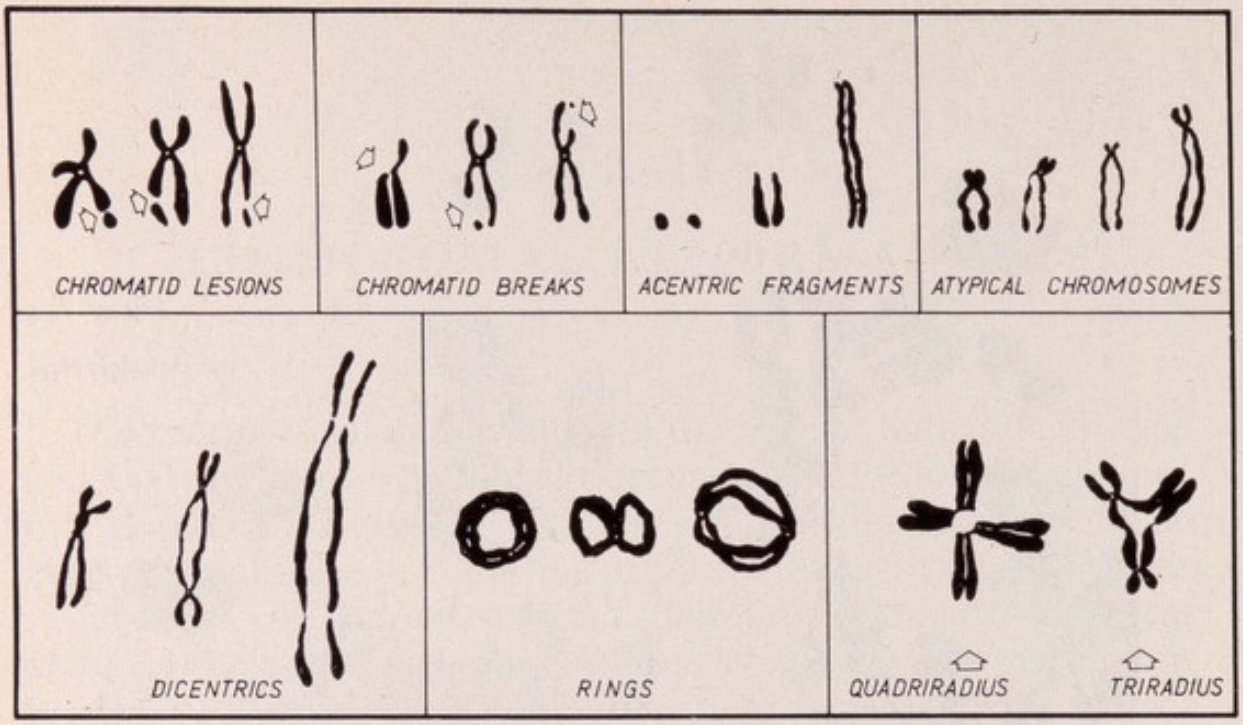

FIG. 8.—Chromosome aberrations. (From Amarose *et al.*, 1967)

Endoreduplication is occasionally found in peripheral blood cultures from some normal subjects but the finding of more than two in any one preparation is more commonly seen in some diseases and following exposure to various agents; the literature has been reviewed by Powsner (1966).

In endomitosis there is failure of the nucleus to divide after mitosis so that a polyploid set of chromosomes is found in the cell but without the characteristic pattern of endoreduplication, the chromosomes being distributed randomly; up to 0·5 per cent of metaphases may be of this type.

Morphological Variations

Normal metaphase chromosomes consist of two chromatids joined at the centromere; abnormalities may be present in one or both chromatids (Fig. 8). Secondary constrictions which are attenuated areas of one or both chromatids can be found on some normal chromosomes but may be accentuated following exposure to certain drugs and chemicals (Cohen and Shaw, 1964). Chromatid gaps are pale achromatic areas whose lengths are not greater than the width of the chromatid, and isochromatid gaps are the same lesions directly opposite each other on both sister chromatids; they may be found in a small proportion of cells from normal

subjects. Chromatid breaks are more complete separations of the chromatid leaving an achromatic area greater than the width of the chromatid and isochromatid breaks are similar but involve both chromatids. They may be found in a few cells from normal subjects. A chromatid fragment is a portion of chromatid completely separated from the chromosome and chromosome acentric fragments result if there is complete separation of both daughter chromatids from the rest of the chromosomes; this rarely occurs in normal cells. Chromatid exchanges are abnormal arrangements of two or more chromosomes formed by the reunion of broken chromatid ends (quadriradius, triradius and chromatid rings). These chromatid aberrations occur in greater numbers of cells in patients with a variety of diseases following exposure to some drugs, chemicals and viruses. Higher concentration of agents which cause accentuation of secondary constrictions may cause gaps and breaks at the same sites on the chromosomes.

Atypical chromosomes are shorter or longer than normal or have an unusual centromere position; they result from deletions, translocations or horizontal divisions. Marker chromosomes are atypical chromosomes found in sufficient metaphases to constitute an abnormal cell population or clone.

A deleted chromosome has lost part of its substance and a translocated chromosome contains all or part of the chromosome material from another chromosome. Di- and tricentric chromosomes have two or three centromeres and are formed by the fusion of portions of two or more chromosomes. Ring chromatids may be formed by the fusion of the chromatids of one arm to form a single ring or by the fusion of both arms to form a double ring. Chromosome rings result from the fusion of the ends of a complete chromosome.

An inversion of part of the chromosome may occur without morphological change. This is called paracentric inversion and the genes lie in reverse order. If the centromere is involved it is called pericentric inversion and results in the formation of an atypical chromosome.

In translocation, the segment of chromosome which is broken away may rejoin at a different site and not produce a morphological change; if, however, a translocated portion joins another chromosome it could lead to the formation of a dicentric or acentric fragment or an atypical chromosome. An isochromosome is one which results from a transverse division of a centromere so

that one daughter chromosome contains all the material from the short arms, and the other, all the material from the long arms. Such chromosomes are always true metacentrics.

The term pseudodiploid is given to those metaphases which have the diploid (2n) number but with one or more atypical chromosomes or additional and missing normal chromosomes, or a mixture of both. Up to 4 per cent of cells from normal cultures may contain structural changes of one type or another (Nasjleti and Spencer, 1968). If morphological aberrations involving the whole chromosome are present in more than one cell from a single slide or more than 5 per cent of cells from a single specimen, disease or exposure to drugs, chemicals or radiation should be suspected.

Chapter V

ACUTE LEUKAEMIA

Introduction

The first chromosomal abnormality in cells from a patient with acute leukaemia was reported by Ford *et al.* (1958); many other patients studied at this time, however, had normal karyotypes (Table II) and it was subsequently realized that some of the PHA stimulated cells in peripheral blood might not have been leukaemic. Since selective growth of cells with a normal karyotype may have occurred, more recent studies have usually been made on bone marrow, although short-term cultures without PHA may also yield leukaemic metaphases in some cases (Baikie *et al.*, 1961*b*; Kemp, 1961; Woodliff, 1962, 1964; Krogh Jensen, 1967*a*; Teplitz, 1968).

Bone marrow cells from untreated patients may also yield only normal metaphases and many of these are almost certainly derived from leukaemic cells (Table III). No constant or typical chromosomal abnormality characteristic of the disease has been found and for diagnostic purposes a negative result is unhelpful. It is possible that an abnormality has remained undetected by current methods of examination and we cannot be certain that there is not a specific chromosomal abnormality in acute leukaemia.

Numerical abnormalities described include aneuploidy, both random and with hypodiploid and hyperdiploid cell lines, polyploidy, endoreduplication and haploidy. Structural aberrations include deletions, marker chromosomes, pseudodiploidy, dicentric chromosomes, lesions and breaks of the chromatids, acentric fragments and an increase in secondary constrictions. These have been found in cells from untreated patients and are therefore related to the leukaemic disorder; however, they are more common following treatment, probably due to the effect of cytotoxic drugs (Weatherall and Walker, 1965; Krogh Jensen, 1967*b*). Abnormalities have been found in all cytological types of acute leukaemia and at all ages from the newborn to the aged (Zussman *et al.*, 1967); some are illustrated in Figures 9–14.

TABLE II

LITERATURE ON THE CYTOGENETICS OF ACUTE LEUKAEMIA TO 1963

Author	Year	Number of Cases	Material*	Number with abnormalities	
				Abnormal Cell Lines	Other Abnormalities**
Ford *et al.*	1958	12 (only 3 with sufficient counts)	BM Culture	1	–
Nowell *et al.*	1958	3	PB Culture	–	1
Nowell & Hungerford	1960*a*				
Baikie *et al.*	1959 1961*c*	22	PB Culture BM Direct	8	6
Bayreuther	1960	5	Not Stated	–	–
Ford	1960*a*	6	BM Culture	4	–
Awano *et al.*	1961	5	BM Direct	1	3
Bottura *et al.*	1961*a*	1	BM Direct	1	–
Bouton *et al.*	1961	1	PB Culture	–	–
Hungerford	1961*a*	7	PB Culture BM Direct (1 case)	–	7
Kemp	1961	1	PB Culture BM Direct	1	–
Kinlough & Robson	1961	8	BM Direct	2	6***
Hungerford & Nowell	1962	9	PB Culture BM Culture BM Direct	4	3
Ruffie & Lejeune	1962	2	PB Culture Skin Culture	2	–
Sandberg *et al.*	1960 1961 1962*a*	22	PB Culture BM Culture BM Direct	15	7
Bottura & Ferrari	1963	1	PB Culture	–	1
Ford & Clarke	1963	1	BM Culture	1	–
Hammouda	1963	1	PB Culture BM Direct	1	–
Reisman *et al.*	1963	1	PB Culture	–	1
Schuler & Kiss	1963	5	PB Culture	–	1***
Thomson	1963	1	BM Direct	–	–
Weinstein & Weinstein	1963	1	PB Culture	1	–

* PB Peripheral Blood
BM Bone Marrow

** Euploid patients without abnormal cell lines but with other numerical and/or morphological abnormalities.

*** Only abnormality in these patients was increased random aneuploidy.

TABLE III

LITERATURE ON THE CYTOGENETICS OF ACUTE LEUKAEMIA SINCE 1964—BONE MARROW STUDIES

Author	Year	Number of Cases	Number with Abnormalities	
			Abnormal Cell Lines	Other Abnormalities*
Fitzgerald *et al.*	1964	11	8	3
Reisman *et al.*	1964*a* 1964*b*	52	30	Present, not quantitated.
Sandberg *et al.*	1964*b* 1968	219	108	Present, not quantitated.
Hayhoe & Hammouda	1965	12	–	5
Kiossoglou *et al.*	1965*b*	60	31	2; also 20 patients with increased random aneuploidy; fuzziness noted in most preparations.
Ponti *et al.*	1965	3	–	–
Krogh Jensen Krogh Jensen & Killman	1966 1967	30	11	18
Kamada *et al.*	1967 1968	37	15	Random aneuploidy in 7 predominantly diploid patients.
Castoldi *et al.*	1968	5	1	4
Engel *et al.*	1968	10	6	–
Khouri *et al.*	1968	7	2	1
Heath *et al.*	1969	10	4	2
Woodliff	1969*b*	22	5	13

In addition to these series, many single cases have been reported.

* Euploid patients without abnormal cell lines but with other numerical and/or morphological abnormalities.

DIAGNOSIS

Aneuploidy

An increase in random aneuploidy suggests a diagnosis of acute leukaemia or allied disorder but its significance is lessened by its lack of specificity and insufficient data about leukaemoid reactions. In cases where diagnosis is in doubt the presence of an abnormal cell line strongly suggests acute leukaemia (Krogh Jensen, 1968; Sandberg *et al.*, 1964*b*). Marked aneuploidy has been described

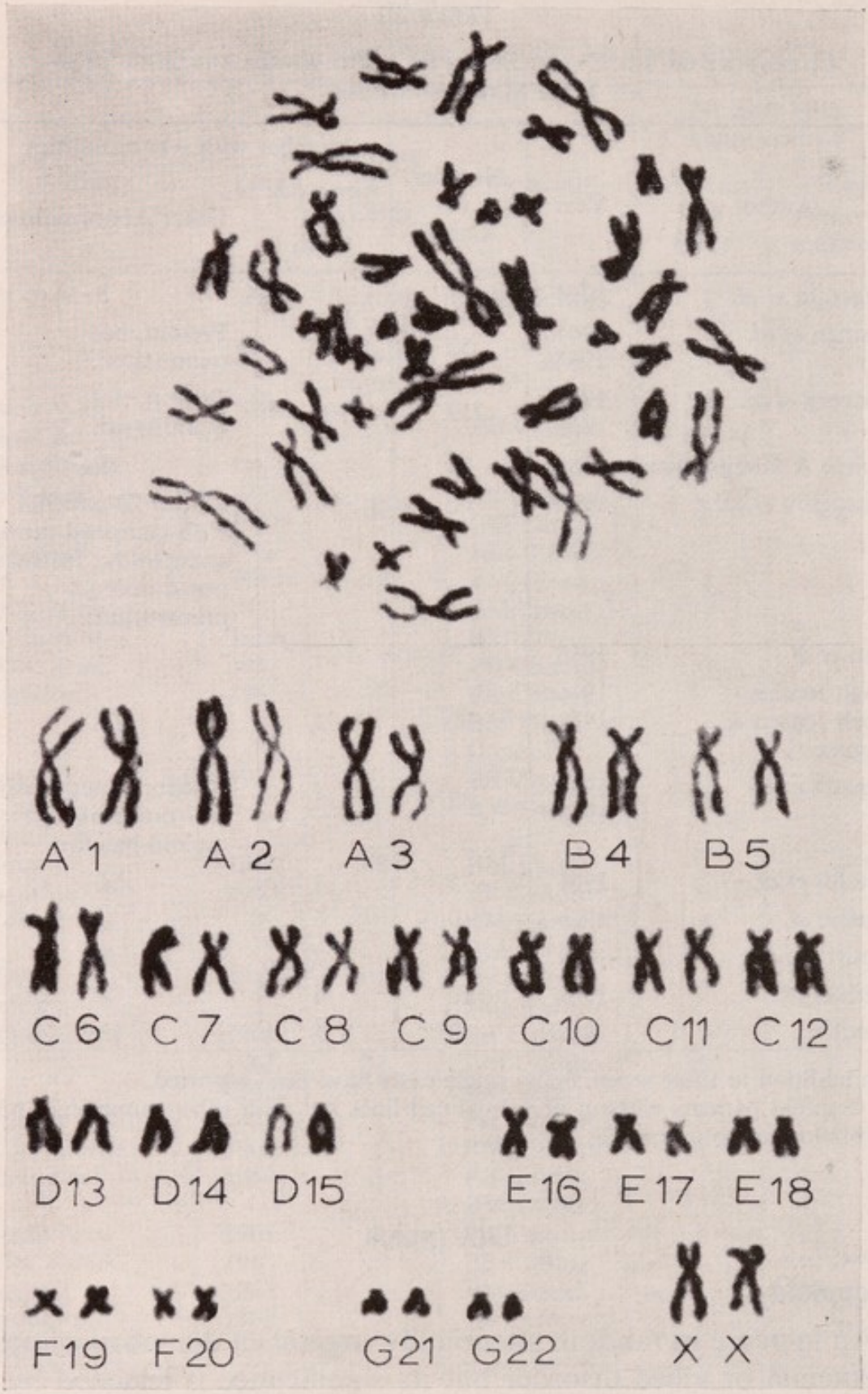

FIG. 9.—Metaphase and karyotype 46,XX from the bone marrow of a patient with acute leukaemia showing "fuzzy" chromosomes (AM200383.0564).

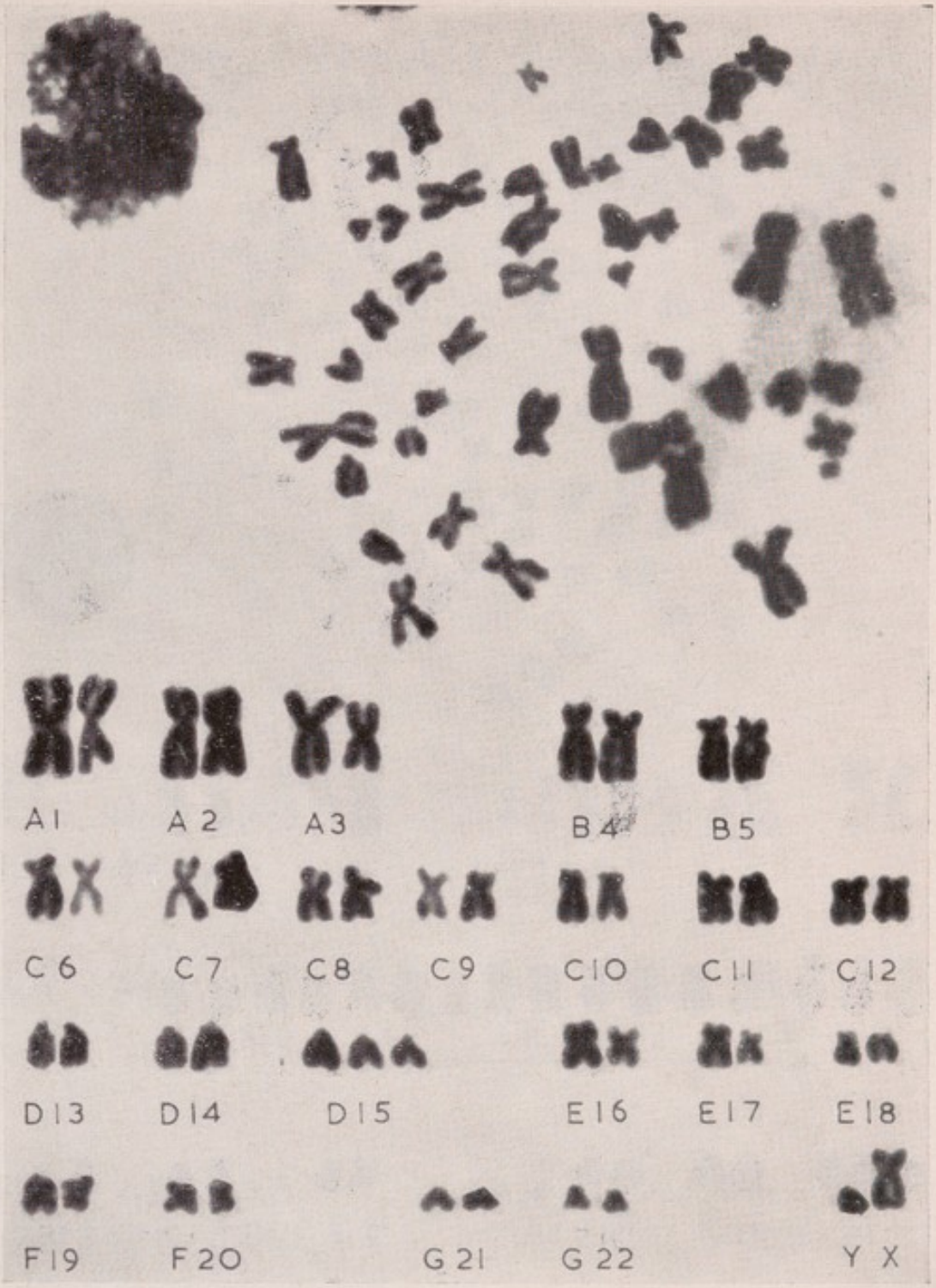

FIG. 10.—Metaphase and karyotype 47, XY, D+ from the bone marrow of a patient aged 31 years with acute myeloblastic leukaemia in relapse following previous treatment with mercaptopurine and prednisone (CA241133.0712M).

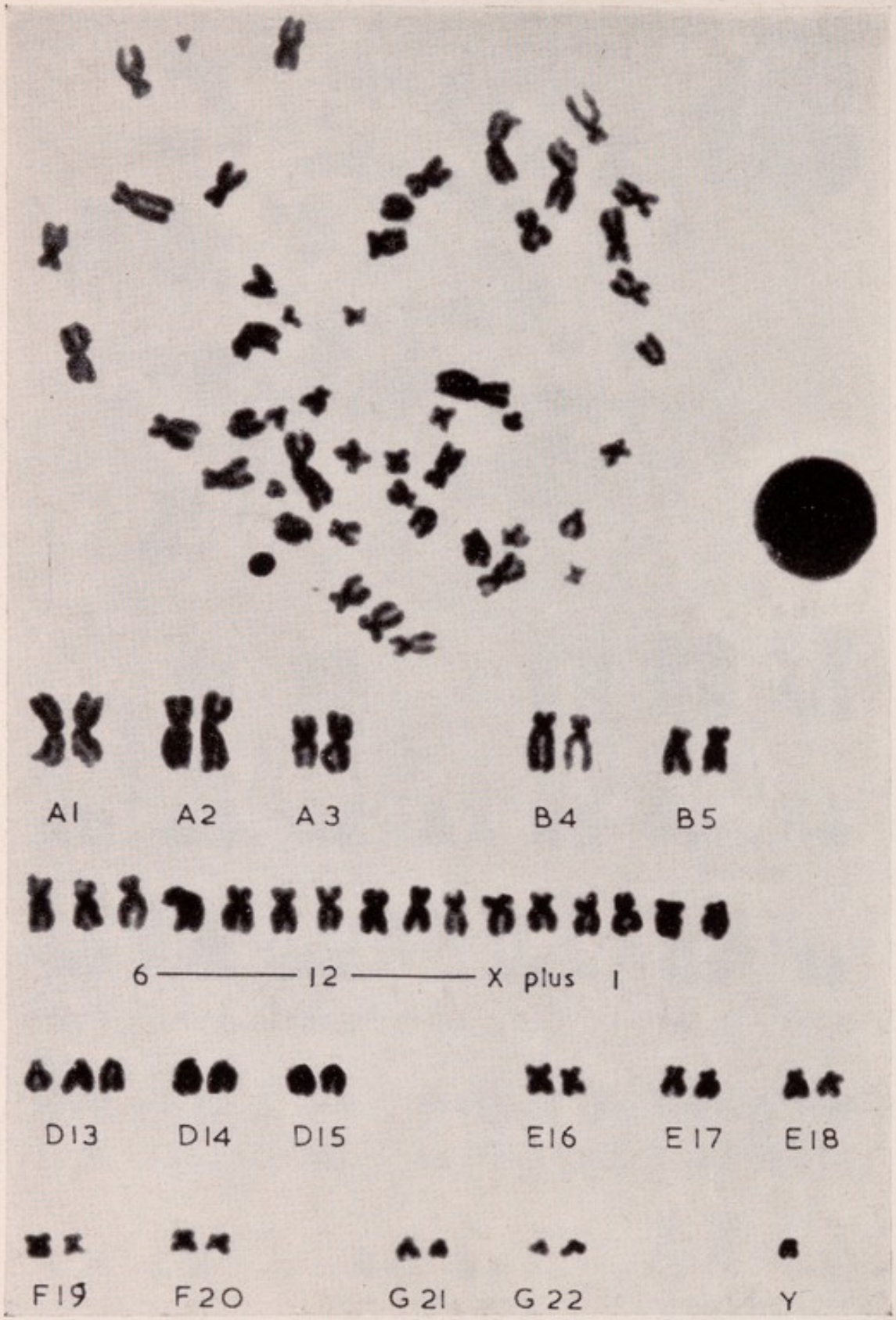

FIG. 11.—Metaphase and karyotype 48,XY,C+D+ from the bone marrow of a patient aged 63 years with untreated acute myeloblastic leukaemia (PH011002. 0899M).

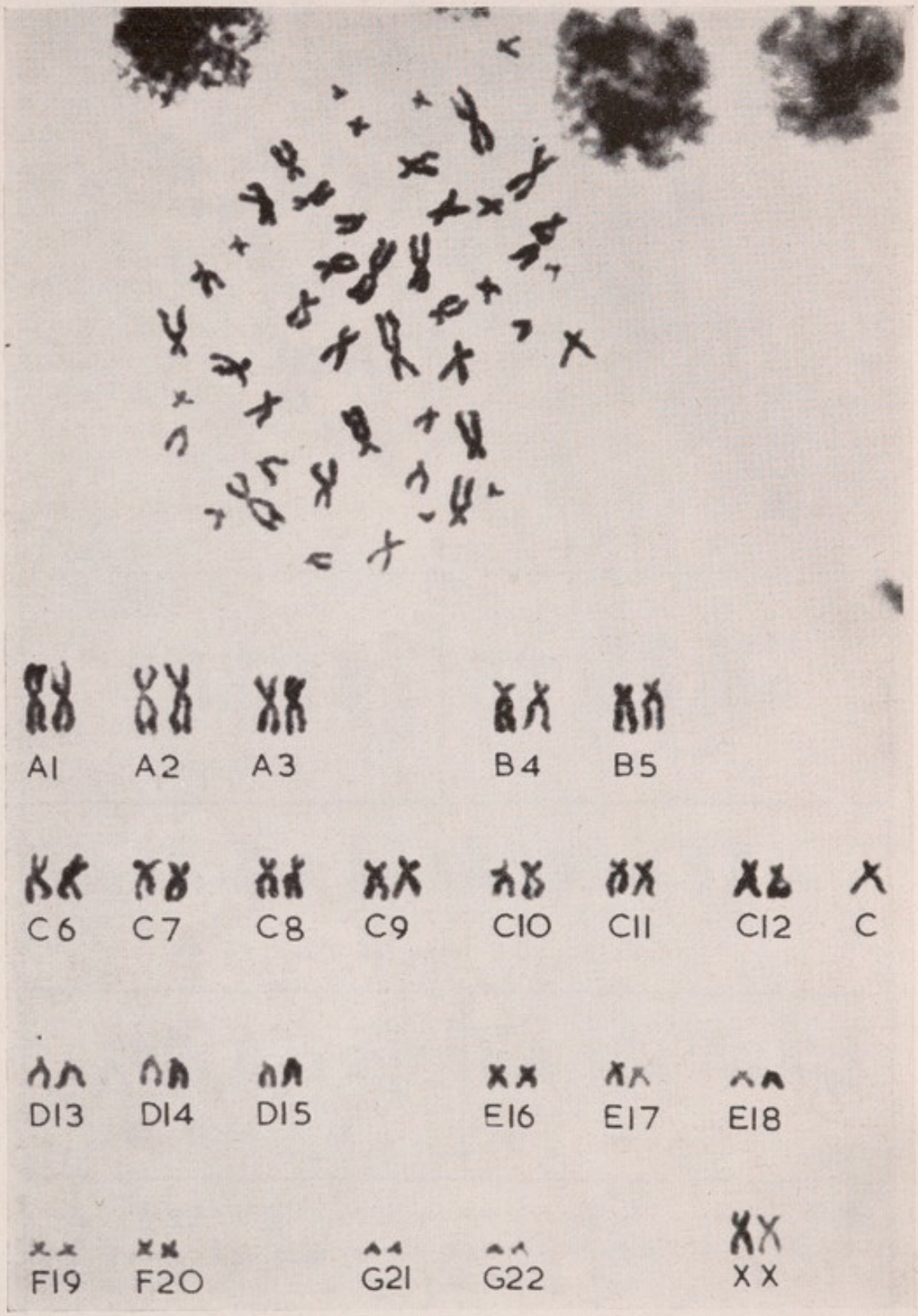

FIG. 12.—Metaphase and karyotype 47,XX,C+ from the bone marrow of a patient aged 46 years with untreated acute myeloblastic leukaemia (WJ140419.0944F).

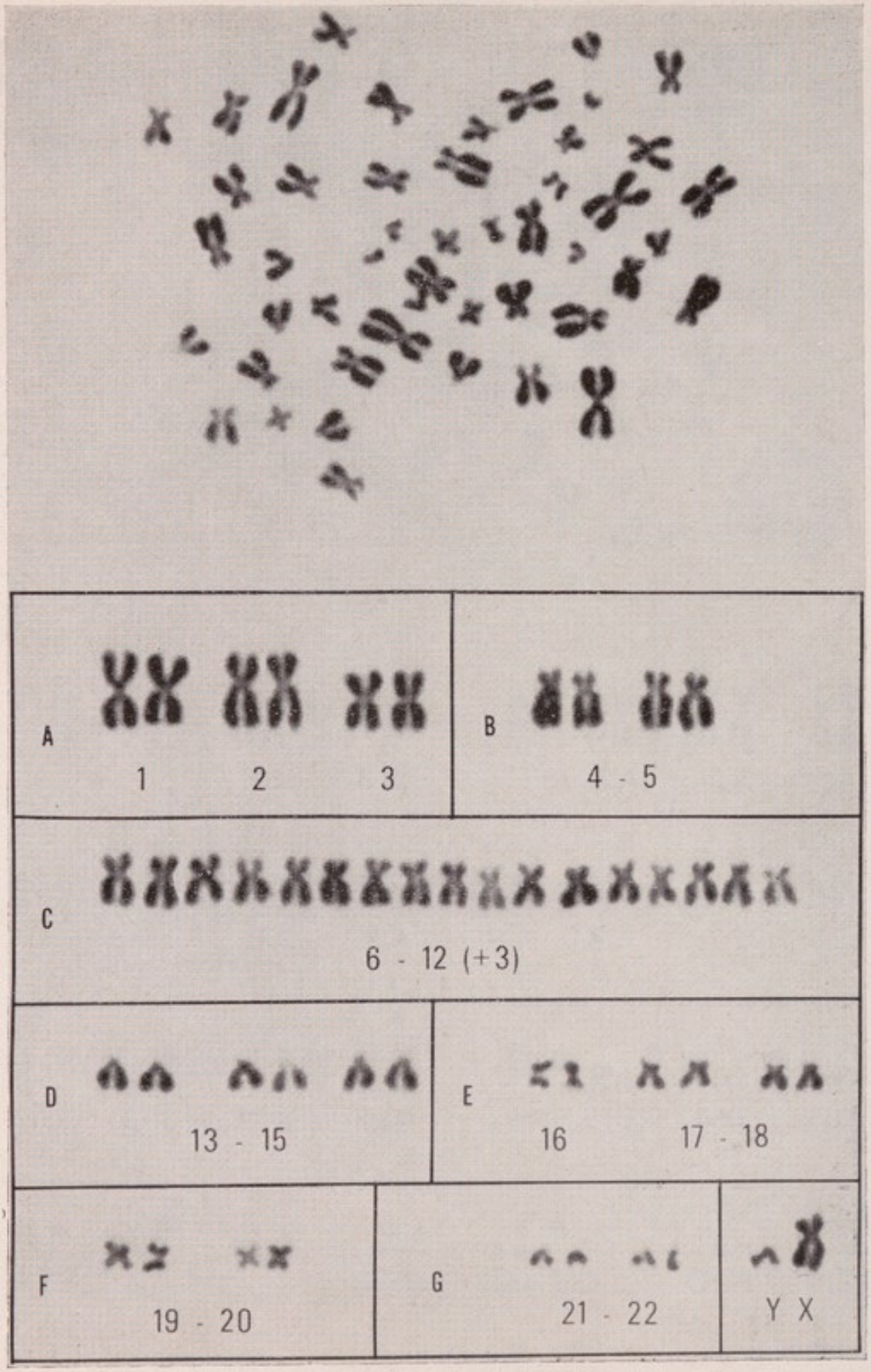

FIG. 13.—Metaphase and karyotype 49,XY,C+++ from the bone marrow of a patient aged 56 years with untreated polycythaemia vera and acute myeloblastic leukaemia (PD020511.1123M).

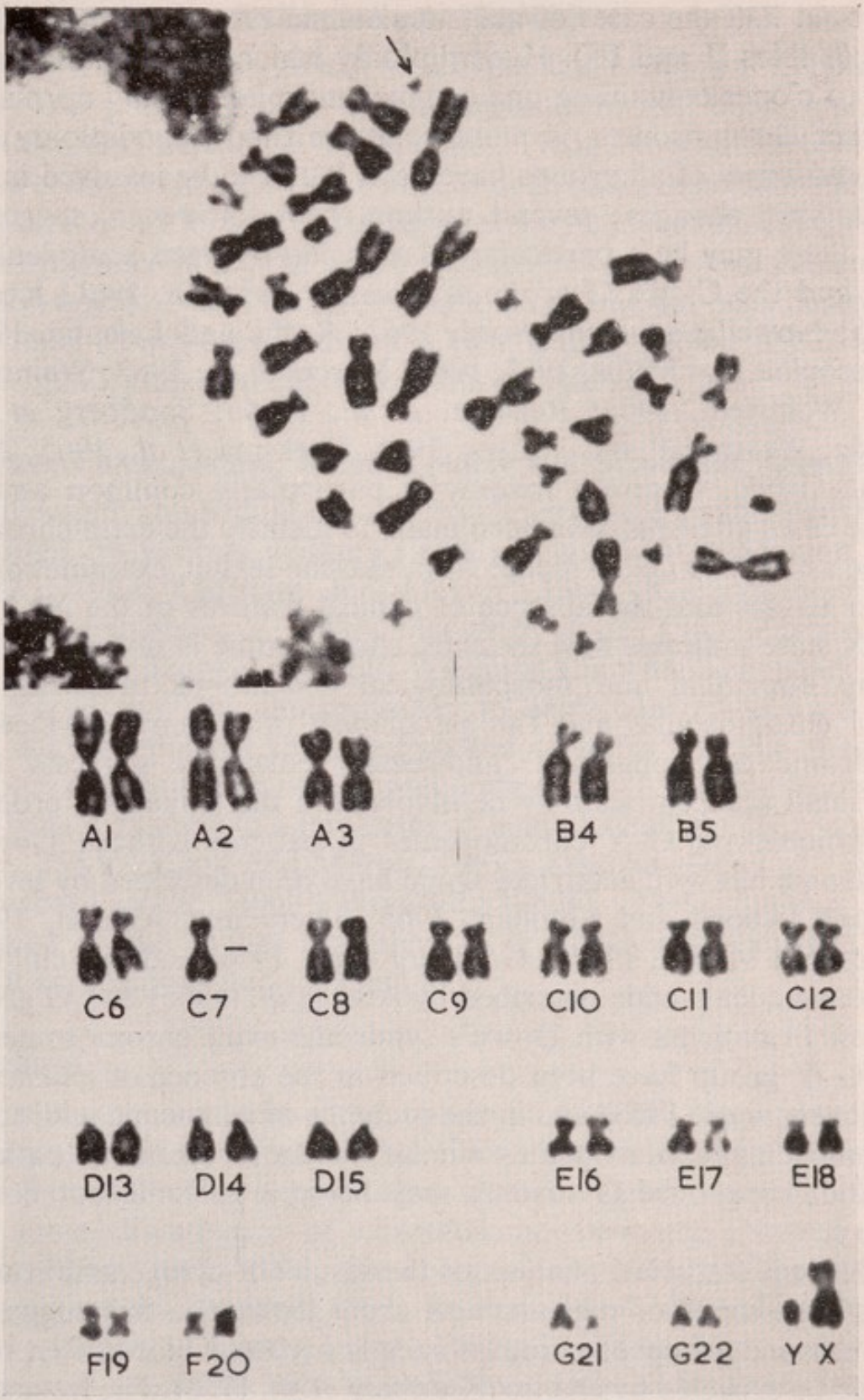

FIG. 14.—Metaphase and karyotype 45,XY,Ph+C−, from the bone marrow of a patient aged 35 years with untreated acute myeloblastic leukaemia (IM130132. 0377M).

in about half the cases of acute leukaemia recorded in the literature (Tables II and III). Hyperdiploidy which may be random or due to clones containing one or more morphologically normal or marker chromosomes, is more common than hypodiploidy and chromosomes of all groups have been found to be involved in the karyotypic changes; several authors have, however, suggested that there may be a particular relationship between acute leukaemia and the C and G group autosomes (Johnson, 1961; Kemp, 1961; Nowell and Hungerford, 1962; Ruffie and Lejeune, 1962; Kiossoglou and Mitus, 1963, 1964; Mercer *et al.*, 1963; Weinstein and Weinstein, 1963; Reisman *et al.*, 1964*b*; Sandberg *et al.*, 1964*a*; Weatherall and Walker, 1965; Leeksma *et al.*, 1965; Awa *et al.*, 1965). C group trisomy is particularly common and in some cases attempts have been made to identify the extra chromosome as a C6 or C9; evidence from nuclear-sexing, examination of other tissues and the absence of clinical features of the XXY or XXX state indicates that the extra chromosome is not an X. The many numerical and morphological aberrations involving the G–Y chromosomes and the association of trisomy G (Down's syndrome or mongolism) and acute leukaemia suggests that the small acrocentrics may be involved in the leukaemic process. Supernumerary G–Y chromosomes in patients without Down's syndrome but with acute leukaemia have been described by several groups (Atkins and Goulian, 1965; Ilbery and Ahmad, 1965; Khan and Martin, 1967*a*; Goldberg *et al.*, 1968) and two children with acute leukaemia described by Awa *et al.* (1965) had G group losses. In patients with Down's syndrome extra chromosomes in the G–Y group have been described in the absence of leukaemia (Valencia *et al.*, 1963) and in the presence of leukaemia additional chromosomal abnormalities similar to those found in patients without congenital G trisomy may be seen in some but not in others.

A recent statistical analysis of the results of cytogenetic studies in a large series of patients with acute leukaemia has suggested that no one group of chromosomes is involved more often than would be expected by chance (Sandberg *et al.*, 1968). The frequency of the C group abnormalities may be related mainly to the large number of medium metacentrics in the karyotype and the involvement of the G group might likewise be fortuitous, but further statistical studies are required before this question can be considered settled.

Polyploidy, Endoreduplication and Haploidy

Cells from some patients with acute leukaemia are hypotetraploid or polyploid and endoreduplication has been reported (Bottura and Ferrari, 1963; Hampel, 1963; Woodliff, 1969*b*). As these changes can also occur in cells derived from normal subjects they are of little diagnostic significance; endoreduplication was seen only once in a large series of acute leukaemias reported upon by Kiossoglou *et al.* (1965*b*). A haploid cell line has been described in a patient with acute leukaemia (Ruffie and Lejeune, 1962; Sorel *et al.*, 1962); this marked abnormality is rare.

Marker Chromosomes, Pseudodiploidy and Structural Aberrations

Structural abnormalities of all the chromosome groups have been reported by various workers (Baikie *et al.*, 1961*b*; Makino and Sasaki, 1964; Chitham and McIver, 1964; Kiossoglou *et al.*, 1965*b*; Leeksma *et al.*, 1965; McCarthy *et al.*, 1965; Zussman *et al.*, 1967; Sandberg *et al.*, 1968). When the abnormality appears with some constancy the chromosome can be recognized as a marker and may be present in an aneuploid clone or in cells with 46 chromosomes, giving rise to pseudodiploidy. A Ph chromosome has been described in a few cases of acute leukaemia (Kiossoglou *et al.*, 1965*b*; Khan and Martin, 1967*c*; Mastrangelo *et al.*, 1967; Grossbard *et al.*, 1968; Woodliff, 1969*b*). These findings add support to the theory that there is a relationship between the small acrocentrics and leukaemia.

Fuzziness

The chromosomes in acute leukaemic cells are often fuzzy and poorly stained; some writers have described them as "ill-defined" and "blurred". This is probably a secondary phenomenon reflecting some disturbance of chromosome structure (Nowell and Hungerford, 1961, 1964; Sandberg *et al.*, 1961, 1962*a*, 1964*b*; Hungerford and Nowell, 1962; Reisman *et al.*, 1963; Kiossoglou *et al.*, 1965*b*; McCarthy *et al.*, 1965). It is found in some non-leukaemic conditions such as pernicious anaemia and also in cells from long term cultures of leukaemic cells. Sandberg *et al.* (1964*b*) found fuzziness particularly marked in acute lymphoblastic leukaemia in both aneuploid and diploid cells; in our experience it is present in most cases of acute leukaemia, occurring more frequently in aneuploid and diploid metaphases and more often in bone

marrow preparations than in those from peripheral blood (Woodliff, 1969*b*).

Cytological Types

An earlier report that aneuploid cell lines from patients with acute lymphoblastic leukaemia are hyperdiploid whilst those from acute myeloblastic leukaemia may be either hypodiploid or hyperdiploid has recently been confirmed by the original observers in a larger series of patients (Sandberg *et al.*, 1964*a*, 1968). This is the general experience, though exceptions do occur, for example Kiossoglou *et al.* (1965*b*) found hypodiploidy in two cases of acute lymphoblastic leukaemia. Reisman *et al.* (1964*b*) suggested that acute myeloblastic and myelocytic leukaemias were associated with less variation from the diploid number than were acute lymphoblastic or stem cell types; this is contrary to our experience, and classification according to cytogenetic findings does not appear to have any advantages over conventional cytological and cytochemical methods.

There is some evidence that abnormal karyotypes may be present not only in leukaemic cells but also in red cell precursors in some cases of acute leukaemia (Krogh Jensen and Killman, 1967); in erythroleukaemia and Di Guglielmo's syndrome, the findings are similar to those in myeloblastic leukaemia. The abnormalities reported include hypodiploidy, non-specific and with loss of specific chromosomes, polyploidy and morphological abnormalities; hyperdiploidy has also been described but is less common (Heath and Moloney, 1965*b*; McClure *et al.*, 1965; Digrado *et al.*, 1964; Baikie *et al.*, 1961*c*; Krogh Jensen, 1966; Durant and Tassoni, 1967; Smalley and Bouroncle, 1967; Crossen *et al.*, 1969; Heath *et al.*, 1969). The literature is well reviewed by Heath *et al.* (1969) who commented that perhaps as many as half the cases with erythroleukaemia contain no discernible chromosome change.

Treatment and Prognosis

Abnormal karyotypes are frequently suppressed and may disappear during treatment but usually reappear during relapse (Sandberg *et al.*, 1964; Reisman *et al.*, 1964; Woodliff, 1969*b*). The return to a normal karyotype may be theoretically desirable but the evidence suggests that complete destruction of an abnormal

clone is uncommon. As the removal of abnormal cell lines does not necessarily improve the prognosis cytogenetic examinations are of little practical value in the control of treatment. Sandberg *et al.* (1964*b*) found that patients with acute lymphoblastic leukaemia and hypodiploidy seemed to respond better to treatment than subjects with diploid cells; generally, however, cytogenetic studies have not proved to be of value in prognosis.

Aetiology and Pathogenesis

Genetic Factors

Reports of several patients with acute leukaemia in the same family have suggested that inherited factors may be important at least in some cases (McPhedran *et al.*, 1969). Studies of twins concordant for acute leukaemia are of special interest because the cytogenetics have been similar for identical twins (Pearson *et al.*, 1963; Hilton *et al.*, 1969) but dissimilar in non-identical twins (Sandberg *et al.*, 1966).

In the cases reported by Pearson, the chromosome analysis was performed on bone marrow aspirates by Dr. J. H. Tjio. Twin "A's" bone marrow was in remission at the time of the study with less than 10 per cent of blasts forms, 126 metaphases were diploid with a normal male karyotype and four were hyperdiploid, one having 64 and three having 65 chromosomes. Twin "B's" bone marrow was in relapse with 90 per cent blasts forms, 20 metaphases were diploid, 19 had 65 chromosomes and four contained between 61 and 64. Both of Hilton's cases were in the active stage of the disease when their bone marrow was examined; 26 metaphases were analysed from twin "M" and two cell lines found, a 46XY and a 47XY with an extra chromosome in the C group; in addition there was random hypodiploidy in a proportion of the cells. Of 22 cells analysed from the bone marrow of twin "J" one was hyperdiploid with an extra C group chromosome, 10 were 46XY, and 11 showed random hypodiploidy.

The fraternal twins described by Sandberg had acute myeloblastic leukaemia, the karyotype of one was hyperdiploid with a mode of 52, whereas the other twin had a hypodiploid cell line containing 45 chromosomes.

Further cases will have to be studied before any definite conclusions can be drawn but the findings suggest a genetic factor is

implicated since similar changes were found in identical and dissimilar changes in non-identical twins.

Congenital Numerical Chromosomal Abnormalities

The association of congenital trisomy 21 (Down's syndrome or mongolism) with leucocyte abnormalities and an increased incidence of acute leukaemia has been well documented (Buckton *et al.*, 1961; Warkany *et al.*, 1963; Kiossoglou *et al.*, 1964*b*) and cytogenetic studies have been carried out on a number of such patients. Several investigators have found no differences between the karyotypes of leukaemic and other patients with Down's syndrome (Wald *et al.*, 1961; Tough *et al.*, 1961; Sandberg, *et al.*, 1961; Thompson *et al.*, 1963; Nowell and Hungerford, 1964); others, however, have reported additional chromosomal abnormalities in some cases. Johnston (1961) found a 48G+C+ cell line in a patient whose cells also contained chromosomal fragments and breaks; after treatment only the usual Down's 47G+ pattern was found. Ross and Atkins (1962) described a female Down's patient with acute myeloblastic leukaemia who had a 47/49 mosaicism and German *et al.* (1962) described a male patient with Down's syndrome who had additional inherited chromosomal abnormalities. Mercer *et al.* (1963) found an extra abnormal chromosome in a child with Down's syndrome and acute myeloblastic leukaemia and Warkany *et al.* (1963) studied a similar patient with a 47G+/54G++D++C++F+ karyotype. Vincent's patient (Vincent *et al.*, 1963) had a 49A++C+F−G+ cell line, and Kiossoglou *et al.* (1963, 1964*b*) reported a very interesting patient with Down's syndrome and acute myeloblastic leukaemia who had many abnormal cell lines with a predominant one containing 51 chromosomes. The patient was one of twins and there was also another pair of twins in the sibship. A possible tendency to non-disjunction was also demonstrated in three healthy members of the family and the authors considered that multiple mitotic non-disjunction abnormalities might be of aetiological importance of the leukaemic process. Additional chromosomal abnormalities are sometimes present before the onset of overt acute leukaemia in patients with Down's syndrome (De Mayo *et al.*, 1967).

Other reports have also indicated that some patients with acute leukaemia may have had other inherited chromosomal abnor-

malities (Miller, 1963; Borges *et al.*, 1967; Twomey *et al.*, 1967; Zuelzer *et al.*, 1968). Two patients with myeloblastic leukaemia have been reported in the same sibship as a patient with XX/XXY mosaicism and acute leukaemia has been described in association with Klinefelter's syndrome (47XXY) and with both 45XO/47XXX and 45XO/46XY mosaicism (Mamunes *et al.*, 1961; Lewis *et al.*, 1963*c*; Baikie *et al.*, 1961*a*). It seems likely therefore that there is an association between chromosomal abnormalities in general and G group abnormalities in particular, and acute leukaemia.

Congenital Morphological Chromosomal Abnormalities

Chromosomal breakage in re-arrangements have been described in Bloom's syndrome, Fanconi's anaemia and ataxia telangiectasia (Bloom, 1966; Bloom *et al.*, 1966*b*; Bloom and Diamond, 1968; Hecht *et al.*, 1966; Sawitsky *et al.*, 1966; Swift and Hirschhorn, 1966; Gmyrek *et al.*, 1968). Since these syndromes are associated with an increased incidence of leukaemia (Garriga and Crosby, 1959) it has been thought that the chromosomal abnormalities might predispose to leukaemic change. In this connection it is of interest that a Ph-like chromosome was found in a cell from a patient with Fanconi's anaemia by Bloom and Diamond (1968); little significance can be placed on this isolated finding but it should stimulate further searches for chromosomal aberrations in this group of patients.

Radiation and Cytotoxic Chemicals

Radiation which can cause a variety of chromosomal aberrations can also lead to acute leukaemia (Buckton *et al.*, 1962*b*) and although a clear progression from typical radiation changes to those found in acute leukaemia has not been demonstrated, it seems likely that radiation is leukaemogenic by virtue of its effects on chromosomes.

Several chemicals and drugs which have been considered potentially leukaemogenic may also cause chromosome damage; they include benzene, phenylbutazone, lysergide and chloramphenicol (Cohen *et al.*, 1967*a* and *b*; Forni and Moreo, 1967; Irwin and Egozcue, 1967; Zellweger *et al.*, 1967; Grossbard *et al.*, 1968; Nielson *et al.*, 1968, 1969; Sato and Pergament, 1968; Garson and Robson, 1969; Hartwich *et al.*, 1969; Woodliff, 1969*b*). Although it is not certain that chromosome damage is implicated

in the progression to leukaemia, this appears to be a reasonable working hypothesis and such chemicals and drugs should be used only when there are no suitable alternatives. The part played by chromosome damage due to the agents mentioned above and to many other drugs, such as streptonigrin and aspirin in carcinogenesis and tetragenesis is not yet clear and further investigations are indicated (Jarvik and Kato, 1968; Zellweger *et al.*, 1967; Hecht *et al.*, 1968).

Virus

Chromosomal abnormalities similar to those seen following exposure to radiation and radiomimetic chemicals may occur in a variety of viral infections both *in vitro* and *in vivo*. There is no evidence that such changes, which may be temporary, progress to acute leukaemia. More suggestive evidence for a viral aetiology comes from epidemiology and from analogy with animal leukaemia and with human Burkitt's lymphoma. Some Burkitt's lymphoma cells contain cytogenetic changes similar to those found in viral infected tissue culture cells but the significance of this is not yet clear (see p. 86).

Conclusions

The significance of cytogenetic changes in the aetiology of acute leukaemia is not yet clear. Visible chromosomal abnormalities cannot be found in some cases and cannot therefore be an aetiological factor in every patient. When they do occur they might be responsible for the cell becoming leukaemic, might be necessary for the progression of the disorder following some other primary stimulus or may merely be secondary changes.

In favour of a primary involvement is the constancy of the change in an individual patient and the general association of acute leukaemia with other conditions in which there are numerical or structural chromosomal abnormalities.

Whether initiating the process or not, chromosomal abnormalities may be necessary for the progression of the disorder in some patients by giving the altered cells a growth advantage over those with a normal karyotype.

In favour of the chromosomal changes being epiphenomena is the fact that they cannot be found in all cases; inherent in this notion is the fact that they are secondary changes resulting from

the leukaemogenic stimulus. However, genetic factors may be important in determining whether visible chromosomal abnormalities are necessary to the leukaemic process, and if so the nature of the change. Some evidence for this is seen in twin studies in which monozygotic twins concordant for acute leukaemia have been found to have similar abnormalities whereas in dizygotic twins with the disease the changes were not the same (Hilton *et al.*, 1969; Sandberg *et al.*, 1966).

Chromosomal abnormalities may be produced by many of the agents discussed above and many of these are also thought to be leukaemogenic, such as viruses, ionising radiation, chemicals and drugs. The changes found include chromatid and chromosome breaks, deletions, translocations, ring formations and so on; actual progression to the leukaemic state has not been observed but there is circumstantial evidence that they may be implicated, either directly or by making the cell more susceptible to leukaemogenic stimuli. Possible multiple factors are involved requiring three or four progressive stimuli to a genetically predisposed cell before it escapes from the normal controlling mechanisms and becomes leukaemic (Schoyer, 1959; Burch, 1964).

Chapter VI

CHRONIC GRANULOCYTIC LEUKAEMIA

Introduction

The report by Nowell and Hungerford (1960*a*, *b*, and *c*) of a characteristic minute chromosome in chronic granulocytic leukaemia and the rapid confirmation of this finding by the Edinburgh group (Baikie *et al.*, 1960) led to extensive studies in other centres. Called the Philadelphia chromosome (Ph^1) after the city in which it was first described (Tough *et al.*, 1961) it is the only abnormal chromosome exempted, for historical reasons, from the rules of nomenclature laid down by the Chicago Conference (1966). Since there will be no Ph^2 the chromosome will be abbreviated here to Ph. It is a small acrocentric chromosome which has lost about half the substance of its long arm; the amount deleted varies but is similar in cells from the same patient (Fig. 15). It belongs to the G group and is considered to be one of pair 21 since "in good preparations of cells containing Ph^1 chromosome two of the three normal small acrocentrics are appreciably smaller than the remaining one" (Court Brown and Tough, 1963). The additional chromosome found in Down's syndrome is also thought to be a 21 (Ford and Wollam, 1968). Where visible, the satellites are said to occur more often and appear slightly larger on pair 21 than on pair 22 and their occasional presence on the Ph chromosome supports the view that it is a 21. Attempts to settle the question by radioisotope uptake studies have been indefinite; some authors have found that the Ph chromosome belongs to the late replicating pair, others have found an inconsistent pattern (Schmid, 1963; Haines, 1965; Sofuni *et al.*, 1967; Goh, 1968*e*). The identification of the small acrocentric chromosomes and their relationship to the Ph, the extra G chromosome in mongolism and the deleted G chromosome in some cases of chronic lymphocytic leukaemia (see p. 76) is the subject of continuing studies. In literature to the end of 1963, most but not all, cases of chronic granulocytic leukaemia reported were Ph positive (Table IV). In our laboratory, the Ph chromosome has been present in all cases and we have been

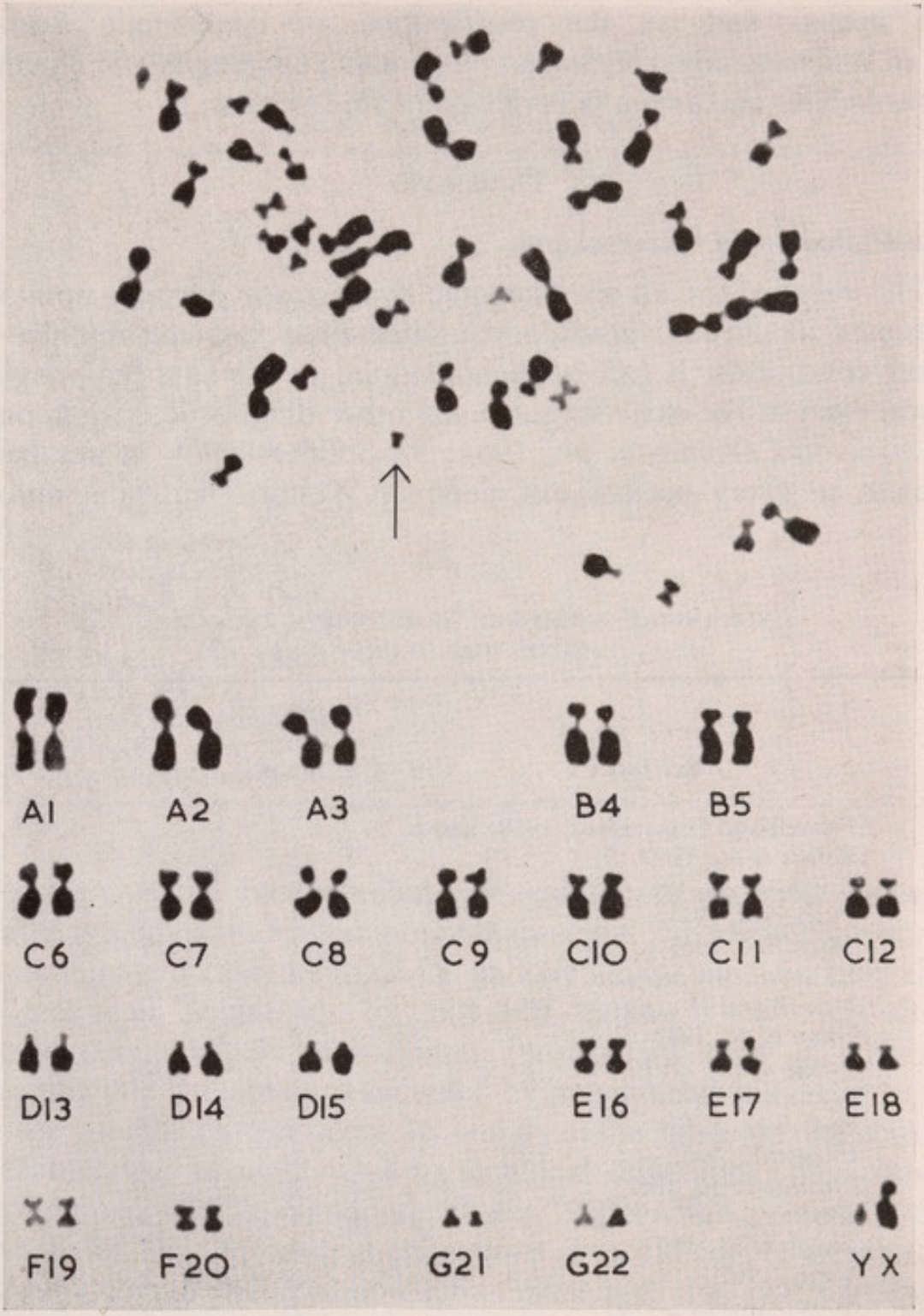

FIG. 15.—Diploid metaphase and karyotype 46,XY,Ph+, from a patient with chronic granulocytic leukaemia showing the Philadelphia chromosome (EF300328.0573M).

interested in its specificity and in the value of cytogenetic studies in diagnosis, prognosis and treatment (Dougan and Woodliff, 1965; Dougan *et al.*, 1967*a* and *b*; Woodliff and Dougan, 1965, 1966; Woodliff *et al.*, 1965, 1966). The literature on these aspects of

the subject and on the contribution of cytogenetic studies to an understanding of the aetiology and pathogenesis of chronic granulocytic leukaemia is reviewed in this chapter.

Diagnosis

The Philadelphia Chromosome

The value of the Ph chromosome in diagnosis depends upon its presence in chronic granulocytic leukaemia and its absence in other conditions. It can be demonstrated in the vast majority of cases, even in the early stages, when other diagnostic criteria may be equivocal (Kemp *et al.*, 1964; Woodliff, 1969*b*). It has been present in every patient examined in Western Australia and a

TABLE IV

Cytogenetics of Chronic Granulocytic Leukaemia
Literature to 1963

Reference	Number of cases examined	Cases Ph positive
Nowell and Hungerford, 1960*a* and *b*	7	7
Baikie *et al.*, 1960	12	8
Adams *et al.*, 1961	4	4
Fitzgerald, 1961*a*	1	1
Hauschka, 1961	11	11
Kinlough and Robson, 1961	4	2
Nowell and Hungerford, 1961	10	9
Ohno *et al.*, 1961	5	5
Tough *et al.*, 1961	18	13
Atkin and Taylor, 1962	1	1
Bosi *et al.*, 1962	1	1
Fitzgerald, 1962	2	2
Fortune *et al.*, 1962	1	1
Sandberg *et al.*, 1962*a*	14	14
Tough *et al.*, 1962	18	18
Yunis, 1962	2	2
Bowen and Lee, 1963	1	1
Fitzgerald *et al.*, 1963	12	12
Jung *et al.*, 1963	1	1
Kundel *et al.*, 1963	1	1
Tanaka *et al.*, 1963	1	1
Tough *et al.*, 1963	27	25
Trujillo and Ohno, 1963	1	1
Whang *et al.*, 1963*a* and *b* Carbone *et al.*, 1963	24	22

In some instances cases may be duplicated in subsequent reports from the same laboratories.

TABLE V

CYTOGENETICS OF CHRONIC GRANULOCYTIC LEUKAEMIA
LITERATURE SINCE 1964
(single case reports excepted)

Reference	Number of cases examined	Cases Ph positive
Hardisty *et al.*, 1964	4	4
Hammouda *et al.*, 1964	4	4
Kemp *et al.*, 1964	5	5
Speed and Lawler, 1964	24	22
Pedersen, 1964*b*	2	2
Krauss *et al.*, 1964	28	16
Goh and Swisher, 1964	10	10
Kiossoglou *et al.*, 1966*b*	25	24
Pedersen, 1966*a*	29	29
Tjio *et al.*, 1966	73	60
Kamada *et al.*, 1967	15	15
Wahrman *et al.*, 1967	2	2
Nicoara *et al.*, 1967	11	11
Goh, 1967*b*	8	8
Elves and Israels, 1967	11	11
Engel *et al.*, 1968	32	31
Woodliff, 1969*b*	36	36

thorough search has revealed no negative cases. This suggests that it is a constant feature of the disease but the literature records some negative cases (Tables IV and V); these may be false due to technical limitations or mistaken diagnosis or true, since the existence of an uncommon Ph-negative type of chronic granulocytic leukaemia is accepted by most authorities. Failure to detect the Ph chromosome in some of the cases in the earlier literature was probably due to technical difficulties (Bayreuther, 1960; Ford, 1960*a*; Sandberg *et al.*, 1960; Awano *et al.*, 1961; and Table IV). In some preparations it is difficult to be certain whether or not the chromosome is present in any one particular metaphase and for this reason cells are sometimes scored as doubtful; with better preparations and more experience this is less of a problem. Negative findings in peripheral blood cannot be considered significant, especially following treatment, because myelocytes may not be present and in fact the Ph chromosome is rarely found in peripheral blood cultures collected during remission (Carbone *et al.*, 1963; Goh and Swisher, 1964; Nowell and Hungerford, 1961); however, it will still be present in the bone

marrow. During relapse short-term non-PHA cultures of peripheral blood as well as direct bone marrow preparations may be made and long-term PHA stimulated cultures may be prepared at the same time as a control. Usually a higher percentage of Ph-positive metaphases can be found in the bone marrow than in peripheral blood and short-term cultures of the peripheral blood give a higher percentage positivity than longer-term PHA cultures which contain an appreciable number of normal metaphases, because in these cultures lymphocytes rather than myelocytes are dividing. Exceptions to these generalities, however, do occur occasionally (Haines, 1965; Woodliff, 1969*b*).

In patients with Ph-negative chronic granulocytic leukaemia there may be a doubt as to the diagnosis; for example, the patient reported by Wahrman *et al.* (1962 and 1963) was probably suffering from myelofibrosis following polycythaemia vera. Others have been "atypical" in some way and should perhaps be considered as examples of other myeloproliferative disorders (Boyd *et al.*, 1965; Nowell and Hungerford, 1962; Sandberg *et al.*, 1962*b*). Some patients, however, appear to be genuine examples of Ph-negative chronic granulocytic leukaemia. Tough *et al.* (1963) reported two patients indistinguishable on clinical and haematological grounds from the average patient with chronic granulocytic leukaemia who had only negative cells in the bone marrow. The patients were considered to belong to a rare subgroup and the authors thought that such negative cases might have a different pathogenesis. Small numbers of Ph chromosome-negative patients have been reported by others (Carbone *et al.*, 1963; Crawfurd and Pegrum, 1964; Engel *et al.*, 1968; Kiossoglou *et al.*, 1966*b*, Speed and Lawler, 1964) but the only large series are those reported from Buffalo and Bethesda (Krauss *et al.*, 1964; Tjio *et al.*, 1966; Whang-Peng *et al.*, 1968). The negative cases differ from the positive patients in having lower leucocyte and platelet counts, in containing a larger proportion of young children under the age of seven, and in responding less well to chemotherapy. Their prognosis is less favourable than the positive patients. They may therefore represent a separate disease and they may not have been diagnosed as having chronic granulocytic leukaemia by some observers. Apparently, a few of the Ph-negative patients are indistinguishable from those who are positive although in most, some clinical feature suggests that they are not typical of chronic granulocytic leukaemia (G. P. Canellos, personal communication,

1967). Both the Bethesda and Buffalo patients were drawn from a wide area and since atypical problem cases tend to be sent to cancer research centres this may be a factor in the relatively high incidence of negative cases. Personal enquiries in several other centres where negative cases have been reported revealed that these patients were atypical in some way.

False positives are less common; they may be due to mistaken identification of the Ph chromosome, to its rare presence in one of the related disorders, or to a mistake in diagnosis.

Most patients with myeloproliferative disorders other than chronic granulocytic leukaemia are Ph negative (Kemp *et al.*, 1962; Nowell and Hungerford, 1962; Sandberg *et al.*, 1962*b*; Solari *et al.*, 1962; Yunis, 1962) and usually when the Ph chromosome has been present the diagnosis of chronic granulocytic leukaemia has either been considered likely or confirmed subsequently (Bowen and Lee, 1963; Cohen, 1967; Kemp *et al.*, 1962 and 1964). A Ph-positive patient provisionally diagnosed as having essential thrombocythemia (megakaryocytic myelosis) by Tough *et al.* (1963) subsequently developed chronic granulocytic leukaemia and died after an acute transformation (A. G. Baikie, personal communication, 1966) and a positive case of megakaryocytic myelosis studied in Western Australia probably suffered from an atypical form of chronic granulocytic leukaemia (Woodliff *et al.*, 1967*a*).

Positive cases have been reported in polycythaemia vera but have either not been fully documented or the unusual chromosome has been subsequently shown to be a small Y (Israels, 1965; Levin *et al.*, 1967, 1968; Goh, 1968*e*; Summitt, 1968).

Heath and Moloney (1965*b*) described a patient with a thrombocytosis and basophilia without splenomegaly in which the Ph chromosome was present; the features were certainly atypical for chronic granulocytic leukaemia but this nevertheless remains the most likely diagnosis.

Eosinophilic leukaemia is a rare condition and a review of the literature reveals that of 11 cases studied cytogenetically the Ph chromosome was present in five (Nowell and Hungerford, 1962; Sandberg *et al.*, 1962*b*; Krauss *et al.*, 1964; Kauer and Engel, 1964; Gruenwald *et al.*, 1965; Goh *et al.*, 1965; Kiossoglou *et al.*, 1966*b*; Elves and Israels, 1967). Insufficient data is given to evaluate the precise diagnosis in all cases but probably the Ph chromosome is present in this disorder which is allied to chronic granulocytic leukaemia and absent in patients with a reactive eosinophilia.

In acute leukaemia the presence of the Ph chromosome usually indicates that the patient is in fact a case of chronic granulocytic leukaemia in acute transformation. In a few cases, however, no evidence of a previous chronic state can be found (Kiossoglou *et al.*, 1965*b*; Tjio *et al.*, 1966; Mastrangelo *et al.*, 1967; Woodliff, 1969*b*).

The Ph chromosome is present in some childhood cases; it was found in cells from a child of two-and-a-half years by Fortune *et al.* (1962) and subsequently by others in children ranging from the age of eight months upwards, with a peak incidence of eight to 12 years (Blake, 1966*a*; Bloom *et al.*, 1966*a*; Wahrman *et al.*, 1967; Neerhout, 1968). Nowell and Hungerford (1962) found cases of "granulocytic leukaemia, atypical, child" to be negative and concluded that they did not have chronic granulocytic leukaemia. Other childhood patients have also been reported as negative (Reisman and Trujillo, 1963; Hardisty *et al.*, 1964; Bloom *et al.*, 1966*a*; Holton and Johnson, 1968). There appear to be two separate diseases sometimes called childhood chronic granulocytic leukaemia, one of which is Ph positive and resembles the adult disease, the other of which is Ph negative, cytologically often chronic myelomonocytic in type and usually associated with thrombocytopenia; it has a worse prognosis and although Ph negative some cases of this type show other chromosomal abnormalities (Reisman and Trujillo, 1963).

Occasional reports of small Ph-like chromosomes in non-malignant conditions (Shaw, 1962; Hall, 1963; Kontras *et al.*, 1966) can be ascribed to small Ys or deleted Gs and since confusion with chronic granulocytic leukaemia is unlikely they are not of diagnostic importance.

Aneuploidy

The increase in random aneuploidy found in several series (Dougan *et al.*, 1967*a*; Goh and Swisher, 1964; Sandberg *et al.*, 1961; Tough *et al.*, 1962; Woodliff, 1969*b*), could be of diagnostic value but its importance is lessened by its lack of specificity and the fact that the Ph chromosome will usually confirm the diagnosis. More notable is the increase in aneuploidy which occurs during acute transformation (see below and Fig. 16); although appreciable aneuploidy may be present in the chronic stage, it increases further during acute transformation. However, it is not possible

to draw conclusions from this generalization because in individual cases aneuploidy up to 50 per cent may be present for many years before death (Woodliff, 1969*b*).

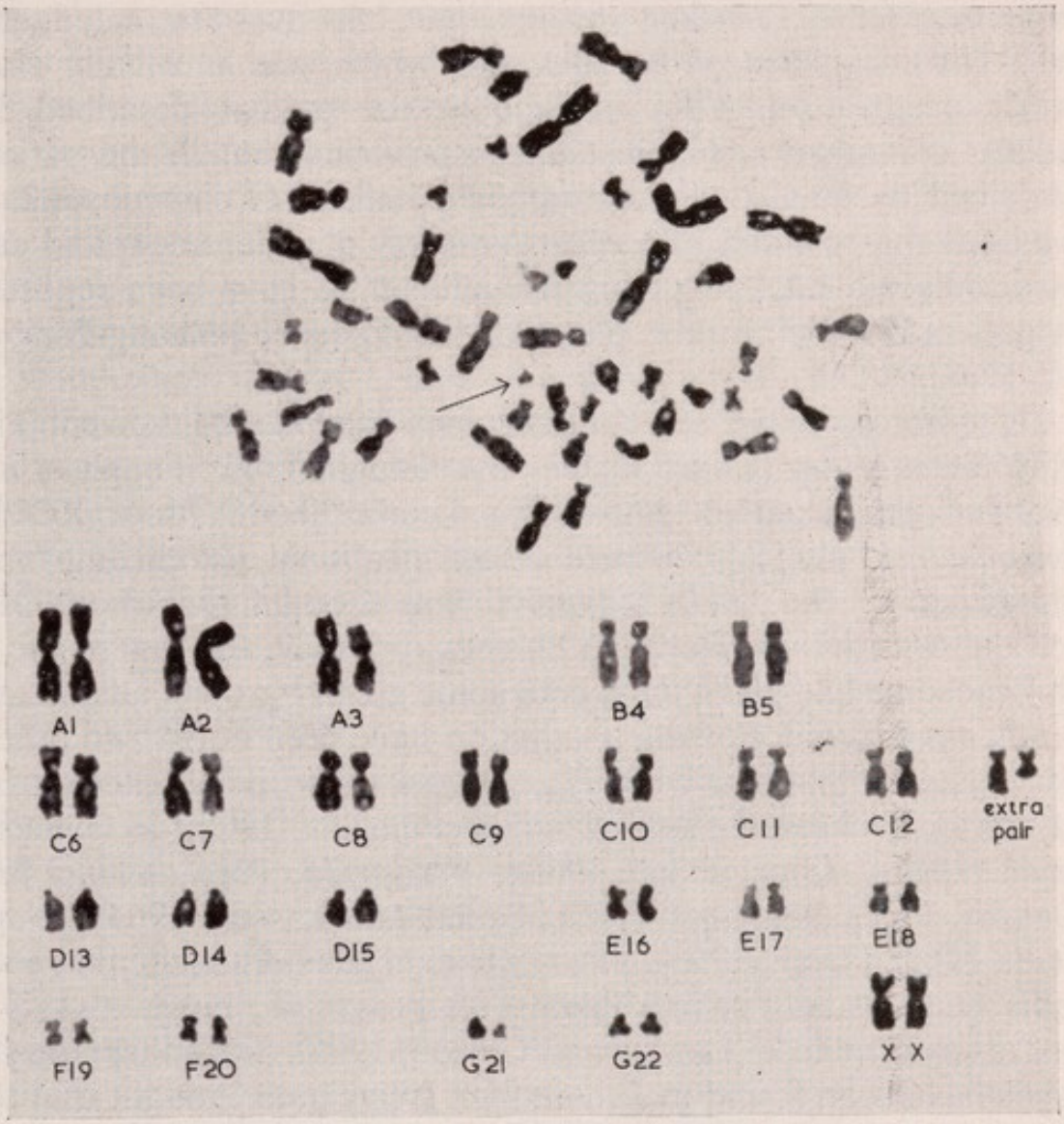

FIG. 16.—Hyperdiploid metaphase and karyotype 48XX,Ph+,2C+, from a patient with chronic granulocytic leukaemia after acute transformation (SM260611.0033F).

Abnormal Cell Lines

As well as an increase in random aneuploidy abnormal cell lines may be found in some patients with chronic granulocytic leukaemia; the karyotypes may be aneuploid or pseudodiploid and the abnormalities are in addition to the presence of the Ph chromosome (Adams *et al.*, 1961; Ford and Clarke, 1963; Levan *et al.*, 1963; Goh *et al.*, 1964; Houston *et al.*, 1964*a*; de Grouchy *et al.*, 1966*b*). Goh's patient had two 47 cell lines, one contain-

ing an extra C chromosome and the other an extra D, both Ph-positive; there was no evidence of acute transformation 11 months after the chromosome study. Houston's patient had a Ph-positive pseudodiploid cell line containing an abnormally large metacentric marker chromosome; she was untreated and in a chronic phase of her disorder which was unusually slow in its progression. The cell line in the patient described by Adams contained a minute chromosome and that in the patient described by de Grouchy contained a marker G chromosome in the bone marrow and skin cells. A variety of other aneuploid and pseudodiploid lines involving the autosomes have been reported in patients in the chronic phase of the disorder (Whang-Peng *et al.*, 1968).

In some cases, the sex chromosomes have been involved. The Edinburgh group (Court Brown and Tough, 1963; Tough *et al.*, 1961) studied a patient whose cells were either XYPh or XOPh; there was no clinical evidence of constitutional sex chromosome mosaicism of the XO/XY type. It was thought that the XOPh cell line was derived from XYPh cells; possibly, the loss of the Y chromosome had given these cells some greater growth advantage. Similar cell lines following irradiation have been noted and others have reported patients whose karyotypes show the absence of one of the G–Y chromosomes (Atkin and Taylor, 1962; de Grouchy *et al.*, 1966*a*; Engel *et al.*, 1965*a*; Fitzgerald, 1966; Lawler and Galton, 1966; Pedersen, 1968*b*; Speed and Lawler, 1964; Tough *et al.*, 1963; Tough, 1965). The suggestion has been made that such individuals usually have a favourable prognosis; however, this is not always the case (Lawler and Galton, 1966). Since these karyotypes have been found mainly in men it has been thought that the lost chromosome has been a Y; Engel (1965*b*), however, has presented evidence that in some cases it may be a G. An Edinburgh patient whose cells were XY Ph + ve, XY Ph − ve, XXY Ph + ve and XXY Ph − ve, had the clinical features of Klinefelter's syndrome; the possible ways in which these cell lines might have been derived are discussed by Tough *et al.* (1961).

Prognosis and Treatment

General Features

Patients in whom the Ph positivity is the only cytogenetic abnormality have a good prognosis; those with an additional

abnormality may do equally well but a documented change to greater aneuploidy is associated with clinical deterioration. Negative cases have a poor prognosis and this is even worse if there are additional abnormalities (Krauss *et al.*, 1964; Whang-Peng *et al.*, 1968). Speed and Lawler (1964) divided their positive cases in the chronic phase into two groups: the first had long remissions following a course of treatment, and the second with a less favourable prognosis enjoyed only short remissions before further therapy was required; cytogenetic studies were of no value in distinguishing the two groups. Similarly, the percentage of Ph-positive cells in the bone marrow does not correlate with survival (Whang-Peng *et al.*, 1968). The relatively good prognosis in some patients with a loss of a single G–Y chromosome has already been mentioned. The association of acute transformation and poor prognosis with marked aneuploidy and with the presence of more than one Ph chromosome is considered further below. Whilst generally valid, prognosis based solely on cytogenetic findings may be misleading in individual cases.

Acute Transformation

In some patients with acute (blast cell) transformation the presence of the Ph chromosome is the only abnormality (Lawler and Galton, 1966; Woodliff, 1969*b*). However, most differ from the earlier chronic phase of the disease in the greater amount of random aneuploidy and in the more frequent presence of abnormal cell lines (Court Brown and Tough, 1963; Engel *et al.*, 1965*b*; Fitzgerald, 1966; Goh, 1967*b*; Kemp *et al.*, 1964; Lawler and Galton, 1966; Nowell and Hungerford, 1962; Pedersen, 1964*b*; Pegrum, 1964; Reisman and Trujillo, 1963; Sandberg *et al.*, 1961; Speed and Lawler, 1964; Whang-Peng *et al.*, 1968; Woodliff, 1969*b*). Hyperdiploidy is the commonest finding but pseudodiploid and occasional hypodiploid lines have been described; it is probable that the blast cells contain these abnormal metaphases because both disappear during remission (Reisman *et al.*, 1964*a*; Garson *et al.*, 1969). The changes are usually in addition to the presence of the Ph chromosome although a few cell lines may be Ph negative (Court Brown and Tough, 1963; Nowell and Hungerford, 1961; Tough *et al.*, 1961). In some patients it is possible to demonstrate a step-wise accumulation of additional abnormalities and this probably contributes to the increasing tempo of the dis-

ease and to its resistance to treatment (Court Brown and Tough, 1963; Fitzgerald, 1966; Pedersen, 1966*a* and *b*, 1969; Pedersen and Videbaek, 1964; Whang-Peng *et al.*, 1968). Often the abnormalities consist of additions or losses of morphologically normal chromosomes but not infrequently marker chromosomes are present; these may be large acrocentrics and submetacentrics, similar to those found in other types of tumours (Goh, 1967*b*; Whang-Peng *et al.*, 1968); all types of chromosomes have been involved but as might be expected the C and G groups are the most frequently affected (Anderson *et al.*, 1968; Engel and McKee, 1966; Fitzgerald, 1966; Lawler and Galton, 1966; Pedersen, 1968*c*, 1969; Teplitz, 1966; Whang-Peng *et al.*, 1968). An increase in aneuploidy may precede any obvious clinical or cytological sign of impending acute transformation (Krompotic *et al.*, 1968); whilst such changes generally indicate acute transformation and a poor prognosis there are occasional exceptions.

Multiple Philadelphia Chromosomes

The presence of more than one Ph chromosome in cells from patients with chronic granulocytic leukaemia has frequently been

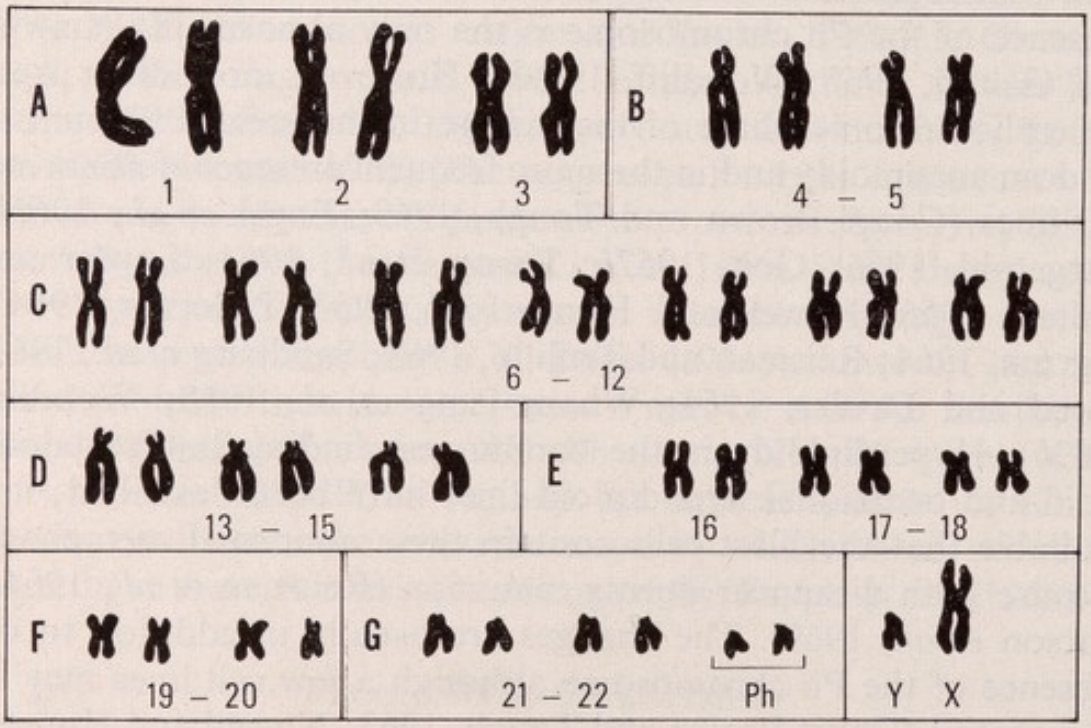

FIG. 17.—Idiogram of metaphase from bone marrow cell containing 48 chromosomes. Group G contains six chromosomes of which two are Philadelphia chromosomes (JV030800.0501M).

TABLE VI

THE MULTIPLE PHILADELPHIA CHROMOSOME STATE
LITERATURE TO 1967

Reference	Case Identification	Cell Lines	Survival in Months
Court, Brown and Tough, 1963	Case 7	48 Ph Ph	—
Hammouda *et al.*, 1964	000049·1 F	46 Ph	10
		46 Ph Ph	
	— 2 M	46 Ph	3
	000030·4 M	46 Ph	—
		46 Ph Ph	
		47 Ph Ph	
Kemp *et al*, 1964	000020·1 M	48 Ph Ph	1
Pedersen and Videbaek, 1964	—	53 Ph Ph	0
de Grouchy *et al.*, 1965	000027·1 F	46 Ph	0
		48 Ph Ph	
		47–51 Ph Ph Ph	
Kiossoglou *et al.*, 1965*a*, 1966*a*	000016·1 M	45 Ph Ph	—
		46 Ph Ph	
		47 Ph Ph	
Ruffie *et al.*, 1965	—	— Ph Ph	—
Schroeder and Bock, 1965	MR000021 F	46 Ph	1
		47 Ph Ph	
Dougan and Woodliff, 1965 Woodliff and Dougan, 1966	JV030800 0501 M	48 Ph Ph	17
Engel and McKee, 1966	000039·1 M	46 Ph	—
		50 Ph Ph	
	000095·2 F	46 Ph	3
		49 Ph Ph	
Erkman *et al.*, 1966 and 1967*a*	000098·1 F	49 Ph Ph	22
	000027·2 F	46 Ph	3
		55 Ph Ph	
		58 Ph Ph	
Lawler and Galton, 1966	DC000030 M	48 Ph Ph	1
	JD000023 M	48 Ph Ph	12
	AJ000033 M	50 Ph Ph	3
Smalley, 1966	4 cases	46 Ph Ph	—
	000002 M	47 Ph Ph	9
Stitch *et al.*, 1966	GS000034 M	48 Ph Ph	3

TABLE VI—*continued*

Reference	Case Identification	Cell Lines	Survival in Months
Streiff *et al.*, 1966*a* and *b*	AF000022 M	46 Ph 46 Ph Ph 47 Ph Ph 48 Ph Ph 48 Ph Ph Ph	0
Tjio *et al.*, 1966	RD000058 M	46 Ph 49 Ph Ph	0
	RH 000046 M	46 Ph 47 Ph Ph	0
Duval *et al.*, 1967	RH000039 M	46 Ph 47 Ph Ph	30
	EB000012 M	47 Ph Ph	18
	RD000029 F	46 Ph 52 Ph Ph	9
Goh, 1967*b*	Case 8 F	47 Ph Ph	2
Hamada and Uchino, 1967	000016·1 F	49 Ph Ph 50 Ph Ph Ph	0
	000015·2 M	47 Ph Ph	0
Khan and Martin, 1967*b*	000035·1 F	44–47 Ph Ph	0
Knospe *et al.*, 1967	Case 1	52 Ph Ph	0
Muldal *et al.*, 1967	000002 F	49 Ph Ph	0

reported (Table VI and Fig. 17); usually two such chromosomes are present in cell lines containing 47 or 48 chromosomes but a variety of other karyotypes have been found. It has been considered by many to carry a bad prognosis with acute transformation either incipient or present and whilst this is general, several cases including one studied by us (Woodliff and Dougan, 1966) have survived for more than a year. An associated prominent peripheral lymphadenopathy noted in several cases (Duvall *et al.*, 1967; Kiossoglou *et al.*, 1965*a*; Knospe *et al.*, 1967) was not present in our patient.

Treatment

An ideal treatment would remove all leukaemic cells from the body; existing therapeutic agents, however, do not achieve this

as the Ph chromosome can usually be demonstrated even when bone marrow hypoplasia is induced (Tough *et al.*, 1962; Carbone *et al.*, 1963; Frei *et al.*, 1964; Speed and Lawler, 1964). Cytogenetic studies are not therefore of practical value in controlling treatment. Both cytotoxic drugs and radiation can induce chromosomal abnormalities (Fig. 18) which may be added to those due to the

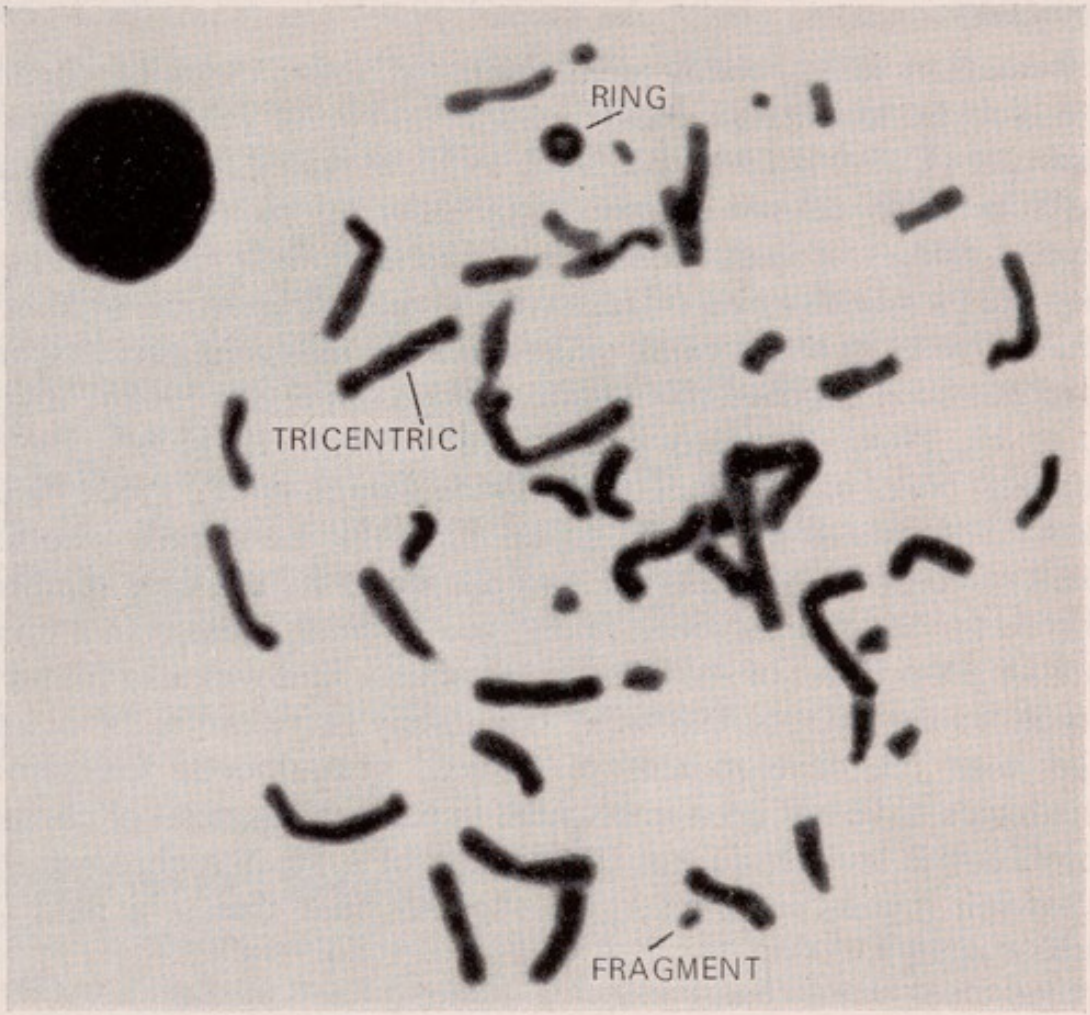

FIG. 18.—Post-irradiation chromosome abnormalities.

disease itself and these changes may lead to the evolution of a new clone of cells, usually hyperdiploid, which are more malignant and possibly drug-resistant (Pedersen, 1968*a*). Acute transformation may follow. However, this should not be taken as a contraindication to treatment because there is ample evidence to show that this improves the quality of life and length of survival. Ph-positive cells can be removed from the blood by treatment but cytogenetic studies are unnecessary as conventional leucocyte

counting techniques will supply all the information which is required to achieve this aim.

The treatment of acute transformation is difficult and remissions are uncommon; however, success may be associated with the suppression of a hyperdiploid cell line (Garson *et al.*, 1969).

Aetiology and Pathogenesis

Radiation

Radiation is an aetiological factor in some cases of chronic granulocytic leukaemia occurring four to twelve years after heavy exposure (Court Brown and Doll, 1957; Heyssell, 1960). Because radiation also causes chromosomal aberrations (Fig. 18) cytogenetic studies of such cases are of interest; with few exceptions they are Ph positive and often have a greater proportion of abnormal cell lines in the chronic stage than are found in patients who have not been exposed to radiation (Buckton *et al.*, 1962*b*; Engel, E. *et al.*, 1964; Gavosto *et al.*, 1965; Sandberg *et al.*, 1962*b*; Tanaka, N. *et al.*, 1963; Tough, 1965; Tough *et al.*, 1960, 1962). Goh (1966) found a small G group chromosome in men who had received total body radiation and discussed its possible relationship to the Ph chromosome. It differed in being present in a much smaller proportion of the bone marrow cells and was also found in lymphoid cells (Goh, 1968*a*). It is unlikely therefore to be associated with the development of chronic granulocytic leukaemia. Chemicals have not been implicated in the pathogenesis of chronic granulocytic leukaemia but the finding of a Ph-like chromosome following ingestion of lysergide suggests that this is a field for further study.

Distribution of the Ph Chromosome

The Ph chromosome is present in most of the bone marrow cells of patients with chronic granulocytic leukaemia. The Edinburgh workers (Tough *et al.*, 1963; Court Brown and Tough, 1963) believe that the great majority of patients have the Ph chromosome in all their bone marrow cells whether in remission or relapse; others have quoted figures of 96 and 100 per cent (Carbone *et al.*, 1963) and the 70 per cent found by our group (Woodliff, 1969*b*) may be too low because some cells were doubtful rather than negative. Negative cells were, however, seen and the

relatively low positivity in some early cases suggests that two myeloid clones may exist in the bone marrow, one with a normal karyotype and the other Ph positive. Support for this view is provided by Whang-Peng *et al.* (1968) some of whose patients had appreciable numbers of negative bone marrow cells.

There is now ample evidence that the Ph chromosome is present in megakaryocytes and normoblasts as well as in myelocytes and therefore probably in the myeloid stem cell (Clein and Flemans, 1966; Fialkow *et al.*, 1969; Frei *et al.*, 1964; Hammouda *et al.*, 1964; Rastrick *et al.*, 1968; Rastrick, 1969; Tough *et al.*, 1963; Trujillo and Ohno, 1963; Whang-Peng *et al.*, 1968*a* and *b*). It has not been demonstrated in fibroblasts growing from skin and other tissues including the bone marrow (Baikie *et al.*, 1960; Baikie *et al.*, 1969; Biscatti and Vaccaro, 1965; Court Brown and Tough, 1963; Engel *et al.*, 1965*b*; Levan *et al.*, 1963; Tough *et al.*, 1961 and personal observations). Negative cells in the bone marrow may be non-leukaemic myeloid cells or occasionally cells of other types, such as lymphoblasts and osteoblasts.

The Ph chromosome occurs in 0 to 100 per cent of cells cultured from the blood of patients with chronic granulocytic leukaemia. Negative findings reflect the state of remission where no primitive myeloid cells are present in the blood (Jung *et al.*, 1963). The Ph chromosome has been found in splenic cells (Spiers and Baikie, 1965*b*) but not in cells obtained from lymph nodes (Whang *et al.*, 1963*a* and *b*).

Origin of the Ph Chromosome

The Ph chromosome is not present in the zygote and probably develops in one or more myeloid precursor cells some years before the clinical onset of chronic granulocytic leukaemia: it has been found in the early and asymptomatic phases of the disease on several occasions (Buxton *et al.*, 1966; Woodliff, 1969*b*).

In favour of it being an acquired rather than an inherited defect is the fact that it is present only in myeloid cells, that it has not been found in monozygotic twins of six patients in chronic granulocytic leukaemia (Table VII) and that it is not usually found in the cells of children born of parents with the disorder. Baikie *et al.* (1969) found the Ph chromosome in the bone marrow cells of a patient with chronic granulocytic leukaemia whose mother had died of the same disease but it was not identified in cultures of

subcutaneous fibroblasts as it might have been if the condition had been inherited. Nor did they detect it in cells from a girl with acute leukaemia whose father had died of chronic granulocytic leukaemia.

However, a report of chronic granulocytic leukaemia in identical twins and a sibling, all said to be Ph positive, suggests that in some cases the development of the chromosomal lesion and the disease may be genetically determined (Tokuhata *et al.*, 1968). No patient has yet been observed to pass from a Ph-negative to a Ph-positive state but this may be expected when large-scale population surveys of bone marrow become possible.

Pathogenesis

The fact that the Ph chromosome is present in cells from nearly all patients with chronic granulocytic leukaemia suggests that it is related to the pathogenesis of the disorder. If it were merely an unimportant by-product of the leukaemogenesis, an epiphenomenon, it would be less constant like the changes found in acute

TABLE VII

IDENTICAL TWINS—ONE WITH CHRONIC GRANULOCYTIC LEUKAEMIA

Reference	Ph Positivity Material	Date
Goh and Swisher, 1965	PB	June 1961
Woodliff *et al.*, 1966	PB	June 1963
Woodliff and Onesti, 1967		December 1963
		August 1964
	BM	June 1965
Jacobs *et al.*, 1966	PB	December 1964
	BM	January 1965
Goh *et al.*, 1967	PB	November 1965
	BM	November 1965
Bauke, 1969	PB	August 1967
	BM	September 1967
Kosenow and Pfeiffer, 1969	BM	November 1967
Gatti *et al.*, 1969	PB	November 1968
(personal communication)	BM	November 1968

PB = Peripheral Blood Culture
BM = Bone Marrow

leukaemia and the secondary changes in acute transformation of chronic granulocytic leukaemia. It is possible that a leukaemogenic

agent or agents may induce malignancy and the Ph positivity in a cell as separate but concomitant phenomena, or the action may be firstly on a chromosome 21 which may become the prime mover of the malignancy which follows. The Ph chromosome-containing cells probably have a growth advantage and gradually take over from normal marrow cells; subsequent evolution, especially towards hyperdiploidy may then be associated with a greater degree of malignancy and lead to acute transformation. It has been suggested that four mutations may be required to produce the parent leukaemic cell but at what stage, if at all, this involves deletion of a G group chromosome is conjectural (Burch, 1965*b*).

Chapter VII

OTHER CHRONIC MYELOPROLIFERATIVE DISORDERS

THE unifying concept of the myeloproliferative disorders in which there is a proliferation of bone marrow cells beyond physiological needs was proposed by Dameshek (1951) and has received much support; generally considered neoplastic, they include acute conditions, such as myeloblastic leukaemia (Chapter V) and chronic disorders, such as chronic granulocytic leukaemia (Chapter VI), polycythaemia vera (PV), myelofibrosis with myeloid metaplasia (MF or MMM), and megakaryocytic myelosis (MM), considered in this chapter.

The use of the collective term, chronic myeloproliferative disorder, was not meant to imply that the separate disorders were similar aetiologically; nevertheless, this requires consideration because some of them merge into one another and patients with the features of one may progress into clear examples of another (Hayhoe, 1960; and Fig. 1). Occasional reports of the Ph chromosome in myeloproliferative disorders other than typical chronic granulocytic leukaemia accords with the unifying concept, but the absence of the chromosome in most patients also indicates fundamental differences. Literature on the cytogenetic studies in these conditions is reviewed in this chapter, an assessment made of their value in diagnosis and treatment and their aetiology and pathogenesis briefly considered in relation to the chromosome findings.

POLYCYTHAEMIA VERA

Polycythaemia vera is a condition in which there is an increase in the patient's red cell mass, and in typical cases in the number of circulating granulocytes and platelets; it may progress to myelofibrosis and less commonly to acute leukaemia. Cytogenetic studies, usually of blood and bone marrow cells, have been examined by several groups and most have been reported as having normal karyotypes (Nowell and Hungerford, 1962;

Traczyk, 1963; MacDiarmid, 1965; Kay *et al.*, 1966; Kiossoglou *et al.*, 1966*b*; Krogh Jensen, 1968). The abnormalities which have been found in some cases are non-specific and no characteristic chromosomal lesion has been described; a normal karyotype does not exclude the diagnosis of this condition. Because many patients who have been studied have received treatment with radioactive phosphorus or with cytotoxic drugs, care must be taken to distinguish chromosomal aberrations which could be due to these from those associated with the disorder itself. An increase in random aneuploidy in PHA leucocyte cultures has been found by a number of workers and in our experience has been significantly different from normal (Onesti and Woodliff, 1968; Woodliff, 1969*b*). Aneuploid cell lines have been described; Kay *et al.* (1966) studied eleven untreated patients, four of whom had normal karyotypes and seven abnormal karyotypes; these included hypodiploid cell lines with various losses of chromosomes involving most groups and in one case a hyperdiploid line with two extra C group chromosomes. Abnormalities following treatment are more common; Kemp studied a patient who had received treatment with radioactive phosphorus and whose bone marrow contained PAS-positive erythroblasts and a 48C++ cell line (Kemp *et al.*, 1962; Anstey *et al.*, 1963). The Philadelphia group (Nowell, 1965; Nowell and Hungerford, 1962) studied five patients treated with radioactive phosphorus and found abnormal cell lines in four, one of whom developed leukaemia; Levan *et al.* (1964*b*) also described a hyperdiploid line in cells from a patient with polycythaemia vera after treatment with radiation to the spleen. Kay *et al.* (1966), in addition to the untreated patients mentioned above, studied 32 patients who had received radioactive phosphorous; of these, 23 showed abnormalities, the commonest derangements being deletions and translocations. Eight had abnormal cell lines and in four of these the abnormality was an apparent deletion of an F group chromosome. There is no clear evidence that chromosomal abnormalities are associated with a poor prognosis or with transformation into myelofibrosis or acute leukaemia (Ford, *et al.*, 1958; Solari *et al.*, 1962). It seems reasonable, however, to suppose that an alteration from a normal karyotype to one showing an abnormal cell line might be associated with some change in the tempo of the disease and that radiation change might lead to leukaemic transformation.

The Ph chromosome has not been found in the vast majority of cases of polycythaemia vera in which it has been sought, including 35 patients studied in our laboratory (Nowell and Hungerford, 1962; Wahrman *et al.*, 1962; Kay *et al.*, 1966; Woodliff, 1969*b*). Kemp reported on a patient with polycythaemia vera who was Ph positive; thought to have a hidden chronic granulocytic leukaemia the patient's condition later progressed into this disorder (Kemp *et al.*, 1962, 1964). Israels (1965) mentioned that the Ph chromosome was present in some cases of polycythaemia vera but these patients have not been fully reported. The Ph chromosome was considered to be present in two brothers with polycythaemia vera but subsequently it was pointed out that the smallest of the acrocentric chromosomes was more likely to be a Y and this was accepted by the authors of the report (Goh, 1968*e*; Levin *et al.*, 1968; Summitt, 1968). Cytogenetic studies therefore are of comparatively little value in diagnosis and the presence of an appreciable aneuploidy or an abnormal cell line although supporting a diagnosis of a blood dyscrasia is of little help in differentiating polycythaemia vera from the other conditions; at present the main value lies in the exclusion of chronic granulocytic leukaemia. Further studies are required on the relationship between additional chromosomal abnormalities and transformation into myelofibrosis and acute leukaemia with their different implications for prognosis and treatment.

MYELOFIBROSIS

In myelofibrosis there is a gradual replacement of the bone marrow by reticulum and fibrous tissues often associated with hyperplasia, in the early stages, of the erythroid, granulocytic and megakaryocytic series of cells. There is also myeloid metaplasia in the spleen and sometimes in other organs; the relationship of this disorder to the other myeloproliferative conditions and reports of abnormalities in the C group of chromosomes makes it cytogenetically interesting (Figs. 19–21). Unfortunately, as the bone marrow is difficult to obtain from these patients there are comparatively few reports in the literature and PHA leucocyte cultures containing lymphocytes and not myelocytes in mitosis are of little value. However, the cells do show a slight increase in aneuploidy which might suggest that some disturbance is taking place (Onesti and Woodliff, 1968). Metaphases can sometimes be

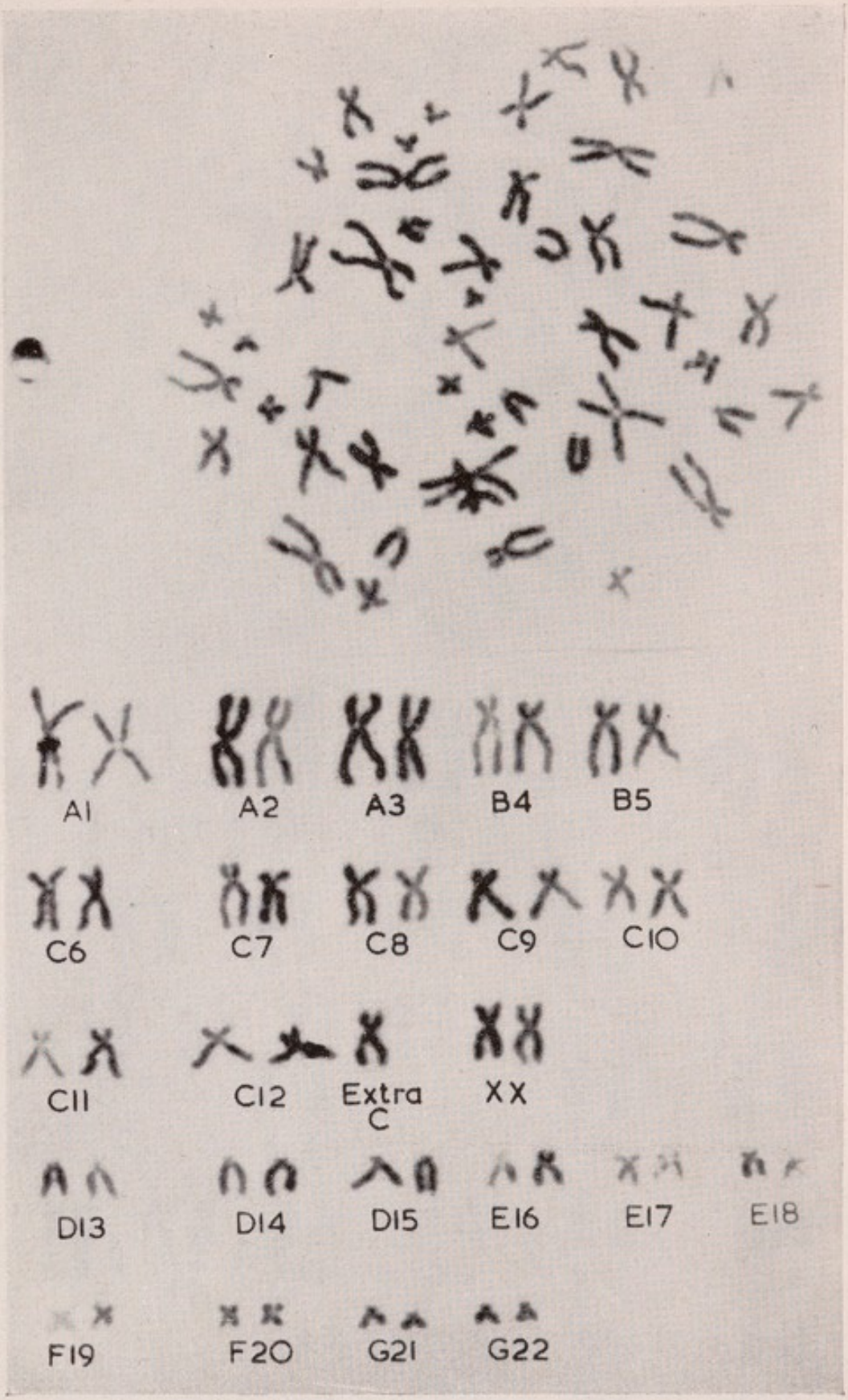

FIG. 19.—Metaphase and karyotype 47,XX,C+, of a cell from a patient with megakaryocytic myelosis (JO280123.0599F).

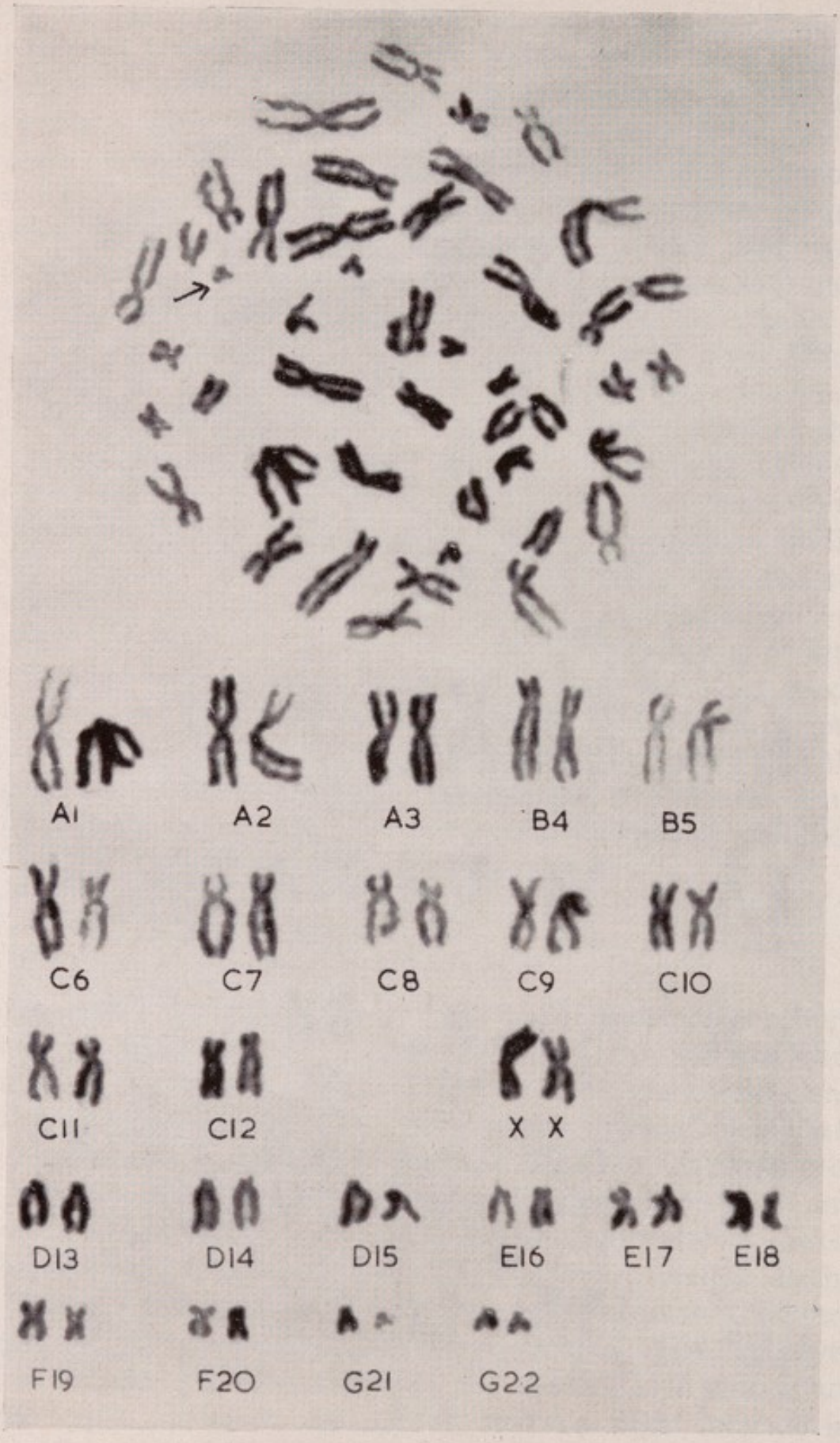

FIG. 20.—Metaphase and karyotype 46,XX,Ph+, from a patient with megakaryocytic myelosis, showing the Philadelphia chromosome (RC031033.0556F).

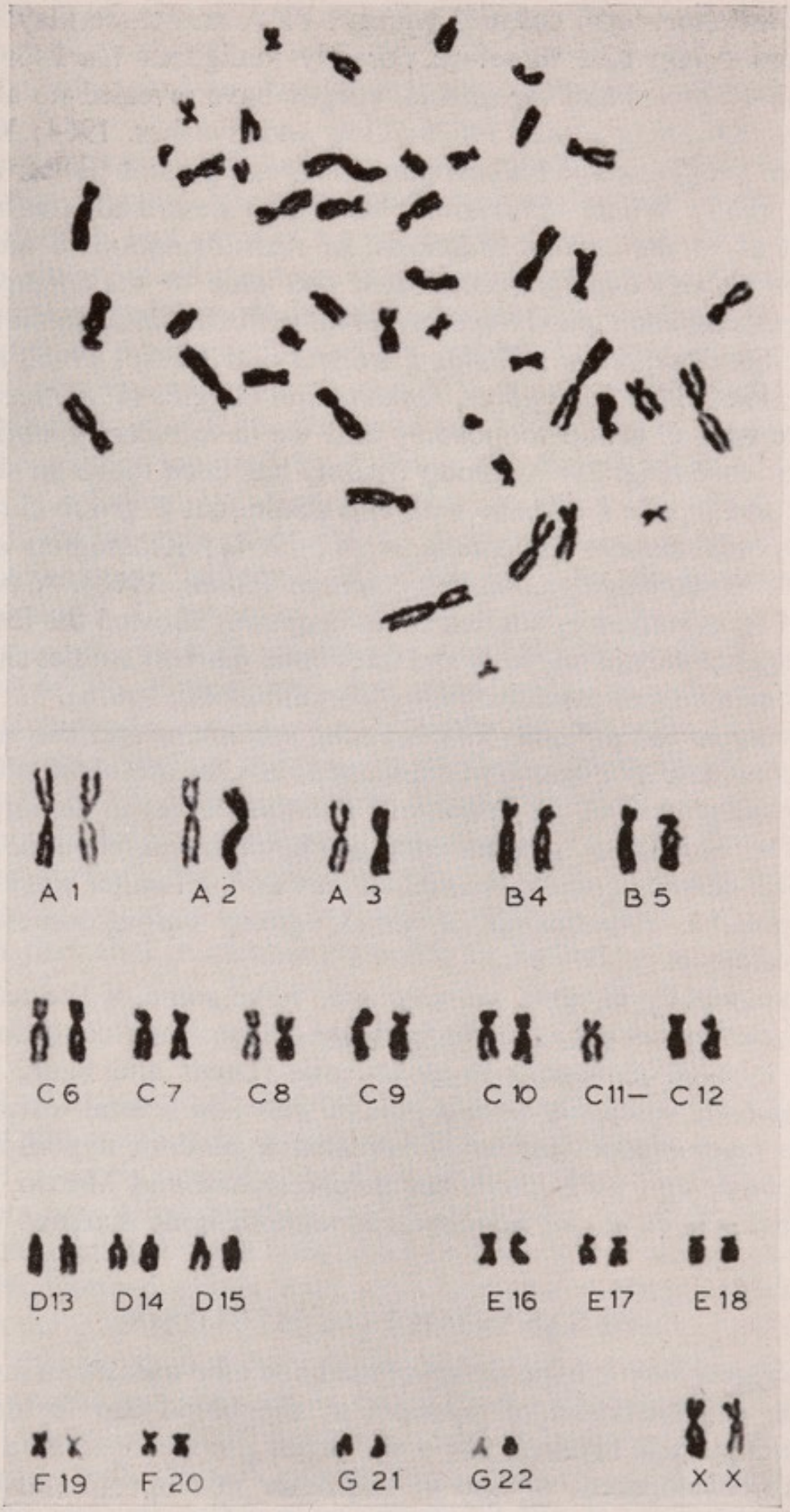

FIG. 21.—Metaphase and karyotype 45,XX,C−, from the peripheral blood of a patient aged 68 with myelofibrosis (MH171002.1043F).

found in short-term cultures without PHA and these may be of myeloid origin and therefore possibly malignant (Jackson and Higgins, 1967). Most cytogenetic studies have revealed no abnormality (Sandberg *et al.*, 1962*b*; Goh and Swisher, 1964; Krogh Jensen, 1968) and the Ph chromosome is not present (Better *et al.*, 1964, 1965). Where aberrations have been described, treatment, doubt as to the correct diagnosis, or transformation to another condition, has usually complicated the issue or the metaphases studied have been few (Nowell and Hungerford, 1962; Solari *et al.*, 1962; Sandberg *et al.*, 1964*a*; Forrester and Lauro, 1966; Better *et al.*, 1964, 1965). However, Jackson and Higgins (1967) reported a case with C group monosomy and we have recently studied a similar case (Fig. 21). C group trisomy has been found in several cases and in one a cell line with two additional C group chromosomes was present (Sandberg *et al.*, 1964*a*; Kiossoglou *et al.*, 1966*b*; Winkelstein *et al.*, 1966; Krogh Jensen, 1968). A patient with C group trisomy studied by us originally showed the features of megakaryocytic myelosis and later bone marrow studies showed some evidence of myelofibrosis (Woodliff *et al.*, 1967*a*).

Owing to the difficulty of obtaining specimens and the lack of any consistent abnormality, cytogenetic studies are of little use in the management of the condition. Their value lies in demonstrating the occasional patient with a Ph chromosome and hence atypical chronic granulocytic leukaemia and in further elucidating the possible relationship of the C group chromosomes with myelofibrosis.

Occasionally patients are seen who have some of the features of myelofibrosis but run a more acute course; their condition has been labelled malignant myelosclerosis (Lewis and Szur, 1963). Cytogenetic studies of such a patient who had several features of acute myeloblastic leukaemia revealed a random hypodiploidy, polyploidy and a Ph-like chromosome (Khan and Martin, 1968) in blast cells from the peripheral blood and bone marrow.

MEGAKARYOCYTIC MYELOSIS

Megakaryocytic hyperplasia of the bone marrow and an increase in the concentration of platelets in the blood are features of megakaryocytic myelosis. In some cases, it appears to be a stage in the development of one of the other myeloproliferative disorders and patients showing the features of the condition may later

develop myelofibrosis or, less commonly, chronic granulocytic leukaemia or polycythaemia vera (Fig. 1). Cytogenetic studies have only occasionally been carried out in this comparatively rare condition.

Four patients with megakaryocytic myelosis (called essential thrombocythaemia by the authors) were studied by Tough *et al.* (1963); three had normal karyotypes and one was Ph positive. The positive patient had been treated with radioactive phosphorus and subsequently developed acute leukaemia, probably a transformation of a pre-existing chronic granulocytic leukaemia (A. G. Baikie, personal communication, 1965). Analysis of three patients by Kiossoglou *et al.* (1966*b*) showed that all had normal karyotypes but the patient reported by Rowley and Blaisdell (1966) had a 48C++ cell line. We examined three patients (Woodliff *et al.*, 1967*a*); the presence of the Ph chromosome in one suggested that the patient was suffering from atypical chronic granulocytic leukaemia; a C group trisomy was found in the second who progressed to probable myelofibrosis and the third with a normal karyotype was subsequently diagnosed as having polycythaemia vera. Cytogenetic examination should be carried out in all patients with megakaryocytic myelosis or thrombocythaemia, as it may prove to be of diagnostic value in revealing one of the other myeloproliferative conditions; this could have prognostic significance because the average survival in polycythaemia vera is longer than that in megakaryocytic myelosis, which in turn is longer than that in chronic granulocytic leukaemia.

Discussion

Not all patients with chronic myeloproliferative disorders fit into one of the nosological entities considered above and transformations frequently occur between the disease categories whilst some of them may also transform into acute leukaemia. Cases that are difficult to classify have been called by a variety of names; Nowell and Hungerford (1962) used such terms as "myeloproliferative syndrome", "chronic granulocytic leukaemia—monocytoid type" and "chronic granulocytic leukaemia—atypical child". The Ph chromosome was not seen in any of these atypical cases of which seven were described; however, four had abnormal cell lines involving the C, D and E groups. Kiossoglou *et al.* (1966*b*) reported four patients with "atypical panmyelosis", two of whom harboured

cells with a C group trisomy; another was Ph positive and therefore probably an atypical case of chronic granulocytic leukaemia. Transformation of megakaryocytic myelosis to chronic granulocytic leukaemia may occur and in such cases the Ph chromosome has been demonstrated. Analysis of a patient with myelofibrosis previously treated with busulphan showed the presence of a Ph-like chromosome (Forrester and Louro, 1966). The deletion may have been due to the cytotoxic treatment or the chromosome may have been a small Y or the case may have been one of atypical chronic granulocytic leukaemia. Chromosomal abnormalities in patients with acute leukaemia following polycythaemia vera have been reported by several groups (Ford *et al.*, 1958; Baikie *et al.*, 1961*c*; Nowell and Hungerford, 1962); as treatment with radioactive phosphorus had been given, it is possible that this had caused the acute leukaemia by damaging the chromosomes. Nowell and Hungerford (1962) also described a case of polycythaemia vera converting to myelofibrosis with a hypodiploid modal chromosome number in the bone marrow; three cells had a missing C group chromosome, three a missing E group chromosome and a single karyotype showed a loss in the G group. This patient, and another with polycythaemia vera and various cytogenetic abnormalities, had received extensive irradiation from radioactive phosphorus. An untreated patient with subacute granulocytic leukaemia following myeloid metaplasia had a C group trisomy and an abnormal chromosome in the D group. C group abnormalities have also been described in chronic erythroleukaemia (McClure *et al.*, 1965) and various blood dyscrasias which might be pre-leukaemic (Freireich *et al.*, 1964*b*; Lawler *et al.*, 1966; Rowley *et al.*, 1966; and see Chapter IX). It appears therefore, that C group chromosomal abnormalities may often be associated with myeloproliferative syndromes; however, as they are the most numerous in the karyotypes the significance of the association is difficult to assess. Further studies are indicated and may lead to a better understanding of the interrelationships and to the so-far obscure aetiology and pathogenesis of these disorders.

Chapter VIII

CHRONIC LYMPHOCYTIC LEUKAEMIA

Introduction

Chronic lymphocytic leukaemia is a common neoplastic proliferation of the lymphatic tissues usually occurring in adults; it is characterized by an increase in lymphocytes in the peripheral blood and their infiltration into solid tissues and organs. It varies from a benign, slowly progressive condition to one which may be fatal within a year. In some cases, a differential diagnosis from a non-neoplastic lymphocytosis may be difficult and there is no single morphological feature which allows a cell to be definitely identified as being leukaemic. The hope that a characteristic cytogenetic abnormality analogous to the Ph chromosome of chronic granulocytic leukaemia might be found, led to an examination of many patients with chronic lymphocytic leukaemia and the report of an abnormal G group chromosome in a family with a high incidence of the disorder gave further stimulus for such studies. The findings are reviewed in this chapter, together with an assessment of the place of chromosome studies in the diagnosis, clinical management and pathogenesis of the disorder.

Diagnosis

Introduction

As cytogenetic abnormalities are more likely to be found in leukaemic than in normal cells, consideration must be given to the selection of suitable tissue. Untreated peripheral blood cultures contain few, if any, metaphases and the responsiveness of leukaemic lymphocytes to PHA is variable, being usually delayed and less marked than that of normal lymphocytes (Nowell, 1960*a*; Woodliff, 1962; Schrek and Rabinowitz, 1963; Bernard *et al.*, 1964*a*, and *b*; Astaldi *et al.*, 1965; Fitzgerald and Adams, 1965; Oppenheim *et al.*, 1965; Ruffie *et al.*, 1966; Schrek, 1967; Lawler *et al.*, 1968). As a result, many of the metaphases in the usual type of culture may be derived from normal rather than from leukaemic

cells. Several methods of selecting dividing leukaemic lymphocytes have been described. Goh (1967*a*, 1968*d*) advocates prolonging cultures for five or six days and Moore *et al.* (1968) recommends that the cell suspension be added to cold medium before incubating at 38°C for 48 hours. Toulouse workers (Colombies *et al.*, 1965; Ruffie *et al.*, 1966) use samples of leukaemic blood frozen to minus 180°C or replace the patient's plasma with normal plasma. Bone marrow specimens from patients with chronic lymphocytic leukaemia usually contain dividing myelocytes, normoblasts and megakaryocytic precursors, but some of the metaphases may be from leukaemic lymphocytes. Lymph node aspirates are a more concentrated source of leukaemic lymphocytes and are preferred by some because there is no contamination with myeloid cells; however, the presence of normal lymphoblasts cannot be excluded (Baserga and Castoldi, 1965).

Aneuploidy and Polyploidy

Most cytogenetic studies of chronic lymphocytic leukaemia have revealed no abnormality (Ford, 1960*a*; Fitzgerald and Adams, 1965; Oppenheim *et al.*, 1965; Ruffie *et al.*, 1966). However, a general random increase in aneuploidy has been reported by some workers, including ourselves (Sandberg *et al.*, 1961; Lawler *et al.*, 1968; Onesti and Woodliff, 1968; Woodliff, 1969*b*) and endoreduplication was noted in the cells of three of 30 patients by Fitzgerald and Adams (1965). These findings are not specific and their value in differential diagnosis would depend on demonstrating their absence in cases of infectious lymphocytosis.

Abnormal Cell Lines

Most interest has centred on the Christchurch chromosome described by Gunz *et al.* (1962); the abnormal chromosome is a member of the G group with deleted short arms (Gp−). It was present in two siblings with the disorder and also in apparently normal members of the family; the father had died of sarcoma of the mediastinum. Subsequently, a third sibling with the cytogenetic abnormality developed the disorder (Fitzgerald and Hamer, 1969). It was suggested that the abnormal chromosome which was present also in the skin cells, and clearly inherited, might predispose to the onset of the disease, but a search for it in other families with chronic lymphocytic leukaemia (Fitzgerald *et al.*, 1966; Bottomley, 1968) and in cases of non-familial leukaemia have been negative.

The significance of the abnormality has been debated (Court Brown, 1964; Abbott, 1966) and whilst it might well be of importance to the family in which it was described, it is not a general phenomenon and visible deletions of the G group chromosome are not a characteristic of chronic lymphocytic leukaemia analogous to the Ph chromosome of chronic granulocytic leukaemia. Autoradiographic evidence suggests that the Christchurch chromosome is a G 22 rather than a G 21, the chromosome involved in G trisomy (Down's syndrome) and deleted in chronic granulocytic leukaemia.

Superficial examination of metaphases may fail to reveal pseudodiploidy detectable by a more careful observation. Goh (1967*a*) analysed 230 metaphases from six patients with chronic lymphocytic leukaemia and found pseudodiploidy in 95 (39 per cent) as compared with six (3 per cent) of 192 karyotypes obtained from normal controls; the abnormal chromosomes encountered included the giant A and D chromosomes, other marker chromosomes and small metacentric chromosomes. Baserga and Castoldi (1965) found a pseudodiploid line in the bone marrow of a patient with chronic lymphocytic leukaemia and Ducos and Colombies (1968) have also reported an abnormal karyotype in two of three patients whose peripheral blood was cultured for six days. The findings of these abnormalities is of great interest, but the large amount of work required makes such tests impractical for routine diagnostic purposes; furthermore, Lawler *et al.* (1968) do not agree that there is a high frequency of pseudodiploid cells in untreated patients.

Prognosis and Treatment

No assessment has yet been made of the relationship between chromosomal abnormalities and prognosis in chronic lymphocytic leukaemia. The patients with Christchurch chromosome do not appear to differ from other cases of chronic lymphocytic leukaemia in the same age group but a study of the family suggests that the so-far unaffected carriers of this abnormality run a risk of developing the disorder. Survival figures are not normally given by those reporting abnormalities but we have analysed data on 27 deceased patients and found no correlation between the degree of aneuploidy and survival. There is no evidence that choice of treatment may be assisted by cytogenetic examination; however, treatment may affect the chromosomes and these abnormalities

must be distinguished from those due to the disorder itself. The pseudodiploidy found by Goh (1967*a*) occurred in both untreated patients and treated patients and is therefore reasonably ascribed to the disorder. Radiation damage similar to that seen in polycythaemia vera is not uncommon in treated cases of chronic lymphocytic leukaemia but the progression to acute leukaemia seen in the former and attributed by some to radiotherapy has not been seen in chronic lymphocytic leukaemia. However, patients with chronic lymphocytic leukaemia usually survive for a shorter time after radiation than do those with polycythaemia vera.

Aetiology and Pathogenesis

The cause of chronic lymphocytic leukaemia is unknown and the chromosome studies have so far made little contribution to the understanding of the pathogenesis; however, changes that have been recorded may be significant and two of these need to be considered further, namely the relationship to the G group chromosomes and the occurrence of pseudodiploidy. Since the visible deletions in the G group chromosomes seen in the families studied in Christchurch are uncommon, they are not of general significance but are of possible importance in the family in which they were described in initiating the leukaemic process. Smaller deletions or condensations of the G group chromosomes which are not ordinarily visible may, however, occur in chronic lymphocytic leukaemia. Fitzgerald (1965*a* and *b*) found that the total length of the G group chromosomes was shorter in chronic lymphocytic leukaemia when compared with controls and thought that this may have been due to the greater degree of spiralization and condensation of some small acrocentrics. We have been unable to confirm this important finding in a study using both measurements of length and area of the G group chromosomes (Woodliff, 1969*b*); this may have been because we were examining cells which were not leukaemic or the methods we used were too insensitive to pick up any small differences. If Fitzgerald's findings could be confirmed they would provide further evidence connecting the G group chromosomes with leukaemia. If pseudodiploidy is as common in chronic lymphocytic leukaemia cells as suggested by Goh, then this may be significant, in that any aberrations of the chromosomes may be associated with leukaemia. The possibility, however, remains that all cytogenetic changes are secondary.

Chapter IX

MYELOMA AND MACROGLOBULINAEMIA

MYELOMA

Introduction

The term myeloma was introduced for localized tumours of the bone marrow and myelomatosis for the generalized condition with the neoplastic cells scattered throughout the bone marrow. It is now recognized that the cells are almost invariably plasmacytes, hence plasmacytoma and plasmacytomatosis are more apt; however, the earlier terms continue in general use. Solitary myelomas are uncommon and are usually found in the bone marrow but occasionally in other reticular tissues; multiple myeloma or myelomatosis is more common, being widespread when the disease is first recognized. Reticular tissues other than the bone marrow may also be involved, abnormalities of the plasma proteins are present and frequently an abnormal protein (Bence Jones) is excreted in the urine. In the rare cases in which myeloma cells are present in the peripheral blood, the term plasma cell leukaemia is appropriate. In the present context, the word myeloma is used to include all these entities. Since it is allied to leukaemia, chromosome studies have been of interest and abnormalities have been found but not in all patients; the findings and their significance are discussed here. Because the condition involves primarily bone marrow, this tissue is usually examined and may yield an abnormal karyotype even when the peripheral blood is normal. However, it must be remembered that many of the cells of the bone marrow may be normal normoblasts, myelocytes and megakaryocytic precursors, so that even if there is appreciable infiltration of myeloma cells abnormal karyotypes may be relatively few. Except in cases of plasma cell leukaemia normal cytogenetic findings might be expected in peripheral blood; in fact, abnormal karyotypes have been described even when they cannot be found in the bone marrow, possibly due to the presence of precursor myeloma cells in the circulation which are stimulated by PHA to divide in culture. Both peripheral blood and bone marrow should

therefore be studied and whenever possible solid plasmacytomas should also be sampled.

Diagnosis

In many cases of myeloma no cytogenetic abnormalities have been detected and since in some of these it seems highly likely that malignant cells were sampled, a normal result cannot be taken as evidence against the diagnosis (Baikie *et al.*, 1959; Richmond *et al.*, 1961; Bottura, 1963; Lewis *et al.*, 1963*a* and *b*; Tassoni *et al.*, 1967; Woodliff, 1969*b*). In some patients non-specific abnormalities, such as increased fuzziness of the chromosomes and a general increase in random aneuploidy occurs (Lewis and McTaggart, 1962; Bottura, 1963; Castoldi *et al.*, 1963; Lewis *et al.*, 1963 *a* and *b*, 1966; Woodliff, 1969*b*). These changes would favour the diagnosis of myeloma if they could be shown to be absent in reactive plasmacytosis, the condition with which it is most likely to be confused; some preliminary evidence suggests that this may be the case but further studies are needed before this point can be settled (Woodliff, 1969*b*). Abnormal cell lines often containing marker chromosomes have been described by several authors (Castoldi *et al.*, 1963; Lewis *et al.*, 1963*a* and *b*; Ritzmann *et al.*, 1966; Coltman, 1967; Das and Aikat, 1967; Houston *et al.*, 1967; Tassoni *et al.*, 1967; Dammacco *et al.*, 1969; Mancinelli *et al.*, 1969). Five of 14 patients studied by Tassoni contained an abnormal acrocentric marker chromosome and Houston found that all three of their patients with IgA myelomatosis and seven out of 14 with IgG myelomatosis also had marker chromosomes resembling those of a large A group member. They suggested that the term MG (standing for monoclonal gammopathy) should be used for these large marker chromosomes. The size of the abnormal chromosome is in the A to B range and the centromere is in the submedian, sub-terminal or terminal region. It may be related to a similar chromosome found in macroglobulinaemia (W —see below) and possibly associated with abnormal protein production (Elves and Israels, 1963). More bizarre changes, both numerical and structural, have been described in the A, B, C, D and G groups in bone marrow and/or peripheral blood (Das and Aikat, 1967; Mancinelli *et al.*, 1969; Dammaco *et al.*, 1969; Ritzmann *et al.*, 1966). Positive findings, especially of a MG marker may be taken as supporting a diagnosis of myelomatosis

but there is insufficient information available to determine whether cytogenetic studies are of value in prognosis or in the treatment of patients.

Aetiology and Pathogenesis

The aetiology of myeloma is not known; the chromosomal changes found are similar to those in acute leukaemia and may have the same significance. However, the presence of a special MG marker chromosome and possible relationship to abnormal protein production is of additional interest.

MACROGLOBULINAEMIA

Introduction

In 1941, Waldenstrom described the case histories of three patients with features of the condition, later accepted as a separate disease entity and now called Waldenstrom's macroglobulinaemia (Waldenstrom, 1944). It is a lymphoproliferative disorder related to lymphocytic leukaemia, lymphosarcoma and myeloma and characterized by anaemia, lymphadenopathy and hepatosplenomegaly and often by a bleeding diathesis. The diagnosis is usually made by demonstrating an increased amount of macroglobulin in the serum but it may be suspected on clinical, histological and cytological findings. Sometimes there may be difficulty in the differential diagnosis from chronic lymphocytic leukaemia and myeloma. Cytogenetic studies have revealed chromosomal abnormalities in some cases and their value in diagnosis and their contribution to the pathogenesis of the condition are reviewed here. Since malignant cells are usually present in the bone marrow it should be sampled whenever possible; blood should also be cultured since abnormalities have been found in the PHA stimulated cells, presumably macroglobulin producing cells or their precursors. Solid tumour tissues should also be studied if available.

Diagnosis

A normal karyotype has been described in several patients with macroglobulinaemia, both in the peripheral blood and in the bone marrow (Pfeiffer *et al.*, 1962; Ferguson and MacKay, 1963; Houston *et al.*, 1967; Woodliff *et al.*, 1968); a normal result therefore does not exclude the diagnosis. An increased aneuploidy may be found and the chromosomes in some cases may look fuzzy

(Houston *et al.*, 1967; Woodliff and Onesti, 1968); these findings are of value in indicating that some abnormality is present and thus leading to further laboratory tests. Abnormal cell lines have been found by several groups; the presence of a marker chromosome was reported in the bone marrow of a single case by Bottura *et al.* (1961*b*) and in the peripheral blood of a single case by German *et al.* (1961). The large marker chromosome was believed to belong to the A group and to have arisen by breaks and reunion in interphase followed by nondisjunction at metaphase. A similar chromosome was found in the karyotypes of the peripheral blood of a patient with macroglobulinaemia studied by Benirschke *et al.* (1962); it was an extra and labelled "W" for Waldenstrom's macroglobulinaemia. Similar markers have been found in other cells from blood, bone marrow and lymph nodes (Pfeiffer *et al.*, 1962; Ferguson and MacKay, 1963; Petit *et al.*, 1968). Houston *et al.* (1967) also found a large chromosome in five or seven patients with macroglobulinaemia and labelled them "MG" appertaining to monoclonal gammopathies as a whole, as they found similar chromosomes in patients with myelomatosis (see above). Although the marker chromosome described by the various groups varies somewhat in the position of its centromere it is usually large and, if present, suggests macroglobulinaemia or myelomatosis as a possible diagnosis.

Aetiology and Pathogenesis

The aetiology of macroglobulinaemia is unknown but there is circumstantial evidence suggesting that the abnormal marker chromosome seen in many of the cases is related to the production of an abnormal protein. Elves and Israels (1963) found an abnormally large chromosome in two patients with a degree of macroglobulinaemia less marked than that usually found in Waldenstrom's syndrome; they suggested that the chromosome conferred upon the cells the ability to produce an abnormal globulin, and thought that the cytogenetic defect may be present for some years before the development of symptoms due to dysproteinaemia. Many possible mechanisms exist whereby the abnormal chromosome may have been produced but whether the same stimulus produces both the abnormal protein production and the cytogenetic abnormality or whether the former is a consequence of the latter is not yet known. The presence of a chromosome abnorm-

ality in relatives of a patient with Waldenstrom's macroglobulinaemia suggests that there may be some genetic basis for the disorder (Brown *et al.*, 1967; Elves and Brown, 1968); however, the discordant findings in uniovular twins, one with a chromosome and protein abnormality and the other without, suggests that it is an acquired disorder (Spengler *et al.*, 1966).

Chapter X

LYMPHOMAS

Introduction

The lymphomas are localized neoplastic disorders of the lymphoid tissues which are histologically divided into several groups, such as follicular lymphoma, lymphosarcoma, Burkitt's lymphoma, reticulum cell sarcoma and Hodgkin's disease. In most, the disease is confined to the lymph nodes or lymphoid tissues, especially in the early stages; bone marrow is not infrequently involved but only occasionally are circulating tumour cells present in the blood. Peripheral blood cultures are therefore only occasionally of value in the investigation of karyotypes of lymphoma cells; bone marrow studies may be more rewarding but preferably lymphoid tissues should be sampled. A further reason for this is that the diseases starts in the lymphoid tissues; bone marrow and blood infiltration may occur later and by then secondary cytogenetic changes may have occurred.

Diagnosis

Chromosome studies of the peripheral blood cultures of most patients have been reported as being normal and the failure to find any abnormality is therefore of no value in diagnosis (Jacobs *et al.*, 1963*b*; Tjio *et al.*, 1963). An increase in random aneuploidy has been present in some cases recorded in the literature and this was found to be significantly increased in our series (Hayhoe and Hammouda, 1965; Woodliff, 1969*b*). The value of this finding would lie in its presence in most of the malignant lymphomas and its absence in reactive lymphadenopathies, a proposition which has yet to be tested. Marker chromosomes and abnormal cell lines have been found in blood in some cases, probably due to the presence of circulating lymphoma cells (Hayhoe and Hammouda, 1965; Sinks and Clein, 1966). Although of considerable interest, these findings are of little diagnostic value because the abnormal cells can be recognized morphologically without recourse to cytogenetic analysis.

Studies of bone marrow material have usually been negative and again a normal finding is of no diagnostic significance (Jacobs, Tough and Wright, 1963). In some cases an increase in random aneuploidy may be present but this is unlikely to be helpful diagnostically (Sandberg *et al.*, 1964*c*; Hayhoe and Hammouda, 1965; Woodliff, 1969*b*). More marked abnormalities and the presence of an abnormal cell line may be found in some cases, indicating an infiltration of the bone marrow with malignant cells (Tjio *et al.*, 1963; Sandberg *et al.*, 1964*c*; Fitzgerald and Adams, 1965).

Biopsy material from lymph nodes and other lymphoid tissues has revealed an abnormality in the vast majority of cases and this is, of course, significant. Direct examination usually reveals few metaphases and some workers prefer to use short-term cultures. Both methods are more complicated than peripheral blood and bone marrow examination and therefore less suitable for routine diagnostic work. The abnormalities which have been described in lymph nodes and to a lesser extent in cases in which the blood and bone marrow are involved, includes an increased "fuzziness" of the chromosomes; an increase in the number and more marked appearance of secondary constrictions, an increase in aneuploidy and the presence of an abnormal cell line. The abnormal cell lines may be aneuploid or pseudodiploid with loss or addition of a normal looking chromosome and in many cases the presence of a marker chromosome. All groups appear to be affected but more particular attention has been paid to the G group chromosomes and a deletion of the short arm of the F group chromosomes which has been found in several cases (Ricci *et al.*, 1962*a* and *b*; Spriggs and Boddington, 1962; Sandberg *et al.*, 1964*c*; Fitzgerald and Adams, 1965; Sasaki *et al.*, 1965; Miles *et al.*, 1966; Spiers and Baikie, 1966 and 1968*a*; Lawler *et al.*, 1967; Bauke and Schoffling, 1968; Kajii *et al.*, 1968). Tetraploid and near tetraploid cells have been frequently recorded. The presence of any of these abnormalities may be taken as presumptive evidence of neoplastic proliferation, although there are few studies of non-malignant lymphadenopathies with which to make a comparison. In some cases of lymphoma normal results have been obtained from lymph nodes and may have been due to sampling only non-malignant cells; however, the possibility that the lymphoma cells themselves have a normal karyotype must also be considered.

L.C.

Classification

The lymphomas are usually classified on histological grounds and this correlates to some extent with the clinical features of the disorders. Attempts to correlate cytogenetic findings and histological classifications are considered here.

Follicular lymphoma.—An increase in aneuploidy in the peripheral blood cultures was noted in two of three patients studied in our laboratory (Woodliff, 1969*b*). Abnormal karyotypes have been described in the lymph node material from several cases; hyperdiploidy with an extra G and F chromosome and cells in a near tetraploid range have been found by Spiers and Baikie (1966, 1968*a*). These findings are not specific for this type but it was noted that a follicular lymphoma of the reticulum cell type contained tetraploid cells as did two of their cases of reticular cell sarcoma; this suggests an association between tetraploidy and malignant reticulum cells.

Lymphosarcoma.—An increase in aneuploidy and other changes, including in some cases an abnormal cell line, may be found in the peripheral blood (Hayhoe and Hammouda, 1965; Woodliff, 1969*b*). Circulating lymphosarcoma cells were present in Hayhoe's patient and the abnormal karyotypes were hyperdiploid. Bone marrow studies have demonstrated both hypodiploidy (Tjio *et al.*, 1963; Spiers and Baikie, 1968*a*) and hyperdiploidy (Sandberg *et al.*, 1964*c*) and lymph node has revealed hypodiploid, hyperdiploid and near tetraploid cell lines. The changes were due to alteration of morphologically normal chromosomes and the presence of marker chromosomes, including fragments (Tjio *et al.*, 1963; Spiers and Baikie, 1966, 1968*a*).

Burkitt's lymphoma.—This special type of lymphosarcoma occurs more particularly in geographically defined regions of Africa and in a younger age group; it is of particular cytogenetic interest because a viral aetiology is strongly favoured by some. Blood and bone marrow studies may reveal no abnormality and lymph node preparations are preferred (Jacobs *et al.*, 1963*b*). These may also be normal but a large variety of abnormalities have now been described, including the presence of hyperdiploid or tetraploid cell lines, the presence of marker chromosomes in both aneuploid and pseudodiploid cells. All chromosome groups may be involved but A2 seems to be more often mentioned and C group chromosomes may show marked secondary constrictions. This is considered by some to be a viral effect (Saksela and Moorhead, 1962;

Clifford *et al.*, 1968; Gripenberg *et al.*, 1969). Cytogenetic status may differ in tumours from the same patient and may also be altered by treatment. It has been suggested that tetraploidy may be associated with recurrence after successful chemotherapy.

Reticulum cell sarcoma.—The peripheral blood may be normal or show an increase in random aneuploidy. Peripheral blood, bone marrow and ascitic fluid may contain abnormal karyotypes belonging to the same clone as those found in lymphoid tissues (Sasaki *et al.*, 1965) which frequently but not always contain abnormal cell lines. These include hypodiploid, pseudodiploid, hyperdiploid and near tetraploid metaphases (Sasaki *et al.*, 1965; Miles *et al.*, 1966; Spiers and Baikie, 1968*a*). There is no close correlation between histology and cytogenetic appearances; however, anaplastic tumours tend to be more often in the tetraploid range.

Hodgkin's disease.—Cytogenetic studies of peripheral blood are normal, show increased aneuploidy or less commonly an abnormal karyotype in circulating Reed Sternberg cells (Sinks and Clein, 1966). Bone marrow is infrequently involved. Lymphoid tissues often show abnormalities but occasionally normal results are obtained and even where visible aberrations are found an appreciable number of normal metaphases may also be present. An increase in secondary constrictions, the presence of marker chromosomes, hyperdiploidy, pseudodiploidy, hypodiploidy and hypertetraploidy have all been described (Spriggs and Boddington, 1962; Galan *et al.*, 1963; Baker and Atkin, 1965; Saksela and Ponten, 1968; Spiers and Baikie, 1966, 1968*a*). Of particular interest is the finding of a marker chromosome, an E with deleted short arms in two cases by Spiers and Baikie (1966, 1968*a*) and in a further case by Millard and Seif (Millard and Seif, 1967; Millard, 1968). Spiers and Baikie suggested that this chromosome be called "M" for Melbourne; a similar aberration, and also deletions with the long arms of the E group chromosome, have been found in other histological types of lymphoma and do not therefore necessarily indicate the Hodgkin's histological type. It might be considered as a lymphoma marker as it has not so far been found as an acquired abnormality in any other condition.

Prognosis and Treatment

Attempts have not been made to correlate cytogenetic findings with survival because of the relatively small numbers of cases

available for analysis. Treatment may suppress an abnormal clone but does not remove it completely and it seems unlikely that chromosome studies will be of much value in the management of lymphoma patients. As in other disorders, care must be taken to distinguish the effects of treatment on chromosomes from those due to the disease itself. A point made by Spiers and Baikie is that although some of their cases had been treated the abnormalities found were similar to those in untreated cases and differed from the aberrations caused by radiation; they could therefore be attributable to the disease process rather than to its treatment.

Aetiology and Pathogenesis

Considerable interest has been shown in the possibility that Burkitt's lymphoma is due to a virus and in support of a viral aetiology of both this and other lymphomas is a finding in some cases of increased numbers and more marked secondary constrictions involving the C 9 and other chromosomes. The changes seen resemble those described in normal human cells transformed by the SV40 virus. The possibility that secondary infection of the material with a leuco or other type of virus cannot, however, be discounted. The essential question in the pathogenesis remains—are the chromosomal abnormalities a part of the neoplastic process or secondary phenomena? Is the escape from a normal karyotype an initiating process in pathogenesis or if it is set off by another factor are the chromosomal abnormalities essential for its continuance? The answers to these questions are not known, however it does seem that in this group of diseases, as in blastic transformation of chronic granulocytic leukaemia, a clonal evolution to a more malignant picture may occur. In a patient studied by Bauke and Schoffling (1968) an increase in the tempo of the disorder was associated with progression to hypertetraploidy and relationship between anaplastic tumours and near tetraploid cells has also been commented on by Spiers and Baikie (1968*a*). The relationship between deleted F group chromosomes and lymphomas found in several cases may be significant but further studies are required. There is some evidence to suggest that lymphomas may originate in a single cell and this could be considered as an argument against viral aetiology because one would expect multiple oncogenic foci. However, a virus may initiate the process only in a preconditioned cell and the whole process derived from the progeny of the single altered cell.

Chapter XI

PRELEUKAEMIA AND LEUKAEMOID REACTION

Introduction

Brief mention has been made of preleukaemia and leukaemoid reaction in previous chapters; here they are considered in more detail.

The preleukaemias are those blood dyscrasias which not infrequently develop into overt leukaemia, that is they are potentially leukaemic. In some cases, leukaemia may already be present but insufficiently developed to allow a definite diagnosis to be made. They can be divided into bone marrow hypoplasias and aplasias, dysplasias of the myeloid tissues and myeloproliferative syndromes (Rowley *et al.*, 1966). Excluded are the leukaemoid reactions in which a leukaemia-like blood picture follows a stimulus such as infection or infiltration of the bone marrow with foreign cells. Leukaemoid reactions revert to normal if the stimulus can be removed; this distinguishes them from the leukaemias which progress, sometimes with temporary remissions, to cause the death of the patient.

Patients with preleukaemia may be cytogenetically normal or abnormal and both these groups may or may not progress to frank leukaemia. A prognosis in an individual is therefore not possible (Rowley *et al.*, 1966; Sandberg *et al.*, 1960). Nevertheless, there is evidence that an abnormal karyotype is associated with an increased risk of developing leukaemia and therefore such patients have a poorer prognosis compared with those whose karyotype is normal (Nowell and Hungerford, 1964; Sandberg *et al.*, 1964*b*; Nowell, 1965). The largest series reported is of 23 patients with "preleukaemia", 13 with a myeloproliferative disorder and 10 with bone marrow depression (Nowell, 1965). Seven of Nowell's patients had a chromosomal abnormality in the marrow and five of these died of leukaemia within three months. Three additional patients with polycythaemia vera treated with radioactive phosphorus had chromosomal aberrations but did not become leukaemic and only

two of 13 without cytogenetic changes in the bone marrow developed leukaemia in a 26 months follow-up.

Although some of the patients may have had leukaemia at the time of the cytogenetic study, these results suggest that a positive finding is related to a poor prognosis. For this reason, and to obtain further experience, cytogenetic studies are indicated in all patients with preleukaemia.

In leukaemoid reactions a similar situation may exist, for whilst one may expect a proportion of patients with leukaemia to be cytogenetically abnormal those with leukaemoid reactions are said to be normal (Nelson, 1968). More case studies however are required and cytogenetic studies should be carried out in all cases where there is a difficulty in differentiating a leukaemoid reaction from a true leukaemia.

PRELEUKAEMIA

Bone Marrow Aplasias

The cells of the bone marrow may be either severely (aplasia) or moderately (hypoplasia) depressed in a variety of congenital and acquired disorders including Fanconi's anaemia, aplastic anaemia, pancytopenia, leukopenia, neutropenia and thrombocytopenia. One cell series (platelet, red cell or granulocytic precursors) may be predominantly involved. Fanconi's anaemia is a congenital condition which includes depression of all the elements in the bone marrow with peripheral blood pancytopenia. Cytogenetic investigations have shown a variety of aberrations, including breaks, gaps, endoreduplication, chromatid exchanges and polyploidy (Bloom *et al.*, 1966*b*; Swift and Hirschhorn, 1966; Bloom, 1966). Acquired aplastic anaemias may be idiopathic or due to known toxic agents, such as drugs, chemicals, or ionising radiations. In many cases, no cytogenetic abnormality has been found and whilst this is a good sign leukaemia may occasionally follow (Nowell, 1965; Rowley *et al.*, 1966; Krogh Jensen, 1968; Woodliff, 1969*b*).

Cytogenetic abnormalities may occur and persist without the development of leukaemia at least for some time; for example, Rowley's patient with aplastic anaemia, possibly due to chloramphenicol, and whose bone marrow contained a cell line 45 XYC–, was in partial remission a year after developing aplastic anaemia. One of Nowell's patients with pancytopenia and a karyotype

containing both extra and missing autosomes and marker chromosomes, showed no change after five months. On the other hand, some of Nowell's patients with karyotypic abnormalities soon died of leukaemia and possibly were leukaemic at the time of the examination. Further experience is obviously required before the role of cytogenetic studies in the diagnosis and prognosis of aplastic anaemia can be fully defined, but the demonstration of an abnormal cell line may indicate a developing or overt malignant process. Many drugs and chemicals which can cause bone marrow depression can also cause chromosome damage and some may be leukaemogenic. Of these, benzene is the best documented (Forni and Moreo, 1967); phenylbutazone has been suggested but only rarely causes chromosome damage in cells exposed to it (Woodliff, 1969*b*). Chloramphenicol, methotrexate, lysergide and other psychotrophic drugs which are thought to cause damage may be preleukaemic (Cohen *et al.*, 1967*a* and *b*; Irwin and Egozcue, 1967; Egozcue *et al.*, 1968; Nielsen *et al.*, 1968; Nielsen *et al.*, 1969; Garson and Robson, 1969).

Bone Marrow Dysplasias

Some blood dysplasias are associated with a decrease in the number of cells in the blood without bone marrow hypoplasia. The term "myeloplasia" has been given to these conditions which are "a group of disorders exhibiting morphologic evidence in the marrow and the blood of disturbed formation, if any, or any combination of the major blood cell lineages (erythroid, granulocytic, megakaryocytic), not characteristic of the aplastic, the myeloproliferative, or the leukaemic states, yet displaying features often intermediate or transitional between these conditions". They include such conditions as refractory hyperplastic anaemia, refractory megaloblastic anaemia, siderochrestic anaemia, atypical myeloid disorder, preleukaemic acute leukaemia and primary splenic hematopenia (Rowley *et al.*, 1966; Heath *et al.*, 1969). Three patients with refractory anaemia and granulocytic hyperplasia of the bone marrow and a low leucocyte alkaline phosphatase were studied by Freireich *et al.* (1964*b*); each had a missing chromosome in the C group. Anaemia and thrombocytopenia had been present for one, two and six years duration; the first two developed overt leukaemia but the other patient's picture remained the same after six years of observation. Six cases of potentially

leukaemic refractory megaloblastic anaemia were studied by Heath *et al.* (1969) and in three, chromosomal abnormalities were found. On follow-up, one with an extra C group chromosome developed overt leukaemia, another was thought to be becoming leukaemic and the third died before further studies could be undertaken.

Sideroblastic anaemia may be idiopathic or secondary; it is characterized by the presence of erythroid hyperplasia with many of the normoblasts containing stainable iron inclusions which form a ring around the nucleus (sideroblasts). A patient with the idiopathic form was studied by Rowley *et al.* who found an abnormal cell line (47C+) in the bone marrow; the patient died without evidence of leukaemia. Six patients with the acquired disorder were studied by de Grouchy *et al.* (1966*c*); in two there was a partial deletion of an F chromosome and in three this group was involved in structural rearrangements. A similar F deletion was also found in a patient with subacute myeloid leukaemia who subsequently developed sideroblastosis. The authors considered the possible involvement of the F group chromosome in erythropoiesis, sideroblastosis and leukaemagenesis.

Myeloproliferative Disorders

The transformation of the chronic myeloproliferative disorders to acute leukaemia has been previously discussed (p. 73). In some cases, normal karyotypes are present and these do not change during transformation to acute leukaemia, and in others, abnormal cell lines are present without transformation (Nowell, 1965; Rowley *et al.*, 1966; Krogh Jensen, 1968). The presence of an abnormal cell line has, however, been considered by some to be a bad prognostic sign and several cases are known in which progression to acute leukaemia has occurred (Nowell, 1965). Probably of more importance is the change in karyotype which may indicate an increase in the tempo of the disease as in some cases of chronic granulocytic leukaemia, when changes additional to the Philadelphia chromosome indicate that acute transformation is imminent (see Chapter VI). Every opportunity should be taken to study further cases so that the value of cytogenetic studies in predicting the conversion to acute leukaemia can be more fully assessed.

LEUKAEMOID REACTIONS

Insufficient cytogenetic studies of leukaemoid reactions have been made and more will be required before the full role of karyotyping in the differential diagnosis from leukaemia can be assessed. Chromosomal abnormalities are said not to occur in leukaemoid reactions (Nelson, 1968) and so cytogenetic studies in such cases may be of value in the differential diagnosis from true leukaemia. However, comparatively few cases have been studied and more evidence is required before full assessment can be made. An acute leukaemoid reaction can be found in a variety of infections, such as tuberculosis, measles and infectious mononucleosis. Carter (1965) made chromosome preparations from six patients in the early stages of infectious mononucleosis and found no qualitative or quantitative abnormalities. Several groups have found abnormalities in long-term cultures from patients with infectious mononucleosis (Tomkins, 1968; Kohn *et al.*, 1968; Carter and Penman, 1969); the significance of these is not known but they may be due to a viral effect. They are presumably not present *in vivo* and so would not be confused with the changes seen in acute leukaemia.

In granulocytic hyperplasias with leucocytosis mimicking chronic granulocytic leukaemia, the absence of the Ph chromosome would favour the diagnosis of leukaemoid reaction. However, atypical forms of granulocytic leukaemia could not be ruled out. Probably in eosinophilia those cases without the Ph chromosome are reactive and those with it leukaemic. Cytogenetic studies are unlikely to be of value in differentiating the lymphocytosis of chronic lymphocytic leukaemia from that of infectious lymphocytosis.

Chapter XII

SUMMARY AND CONCLUSIONS

THE advances in techniques of cell culture and the preparation of metaphases led in the 1950s to an upsurge of interest in cytogenetics and to important advances, including the establishment of 46 as the diploid number of human chromosomes.

The association of congenital syndromes, such as Down's (mongolism), Turner's and Klinefelter's, with numerical chromosomal anomalies was recognized; attention was given to other congenital abnormalities and acquired abnormalities, including neoplasms. The first chromosome abnormality in human leukaemia was described in 1958 and the Ph chromosome, a specific marker for chronic granulocytic leukaemia, was discovered in 1960. These findings stimulated a great deal of work on leukaemia cytogenetics and now at the end of a decade the position can be reviewed, as existing techniques of examination with the light microscope are unlikely to yield much further information. Further advances are more likely to come from a more detailed study of chromosome morphology by electron microscopy and other techniques, and from studies which combine chromosome metabolism and morphology, such as autoradiography. A greater quantity of information may also be obtained once automated techniques are fully developed and increased precision of chromosome measurements may reveal changes not seen by simple inspection.

Cytogenetic studies in the leukaemias, lymphomas and allied disorders are more likely to be rewarding if the actual malignant cells are sampled and this may not always be easy. Phytohaemagglutinin in leucocyte cultures may stimulate non-malignant lymphocytes rather than leukaemic cells, resulting in only normal metaphases being present in peripheral blood from patients with acute leukaemia, chronic granulocytic leukaemia, or other myeloproliferative disorders; therefore a short-term culture without PHA may be preferable. This applies also to some of the other conditions, but PHA cultures may yield abnormalities in some of the lymphoproliferative disorders and myelomatosis; possibly an

atypical or precursor malignant cell in the blood is stimulated into mitosis. Bone marrow is a more suitable tissue in several respects; it contains dividing cells and direct methods without stimulation or culture may yield metaphases; furthermore, it is usually the primary site of malignant change in the myeloproliferative disorders and myelomatosis.

In the lymphoproliferative disorders, including the lymphomas, lymph nodes and other lymphoid tissues, such as the spleen, are a better source of abnormal cells. Unfortunately, such tissue is more difficult to obtain than blood and bone marrow.

Cytogenetic studies have a definite, albeit limited, part to play in the diagnosis of leukaemias. In acute leukaemia, a normal result is unhelpful; fuzziness of the chromosomes and an increase in aneuploidy suggests the diagnosis and the presence of an abnormal cell line is almost diagnostic of leukaemia or a related malignant process. In chronic granulocytic leukaemia the presence of the Ph chromosome is, with very rare exceptions, diagnostic and its demonstration is becoming an accepted necessary part of the clinical management of such patients. The other disorders do not have specific chromosomal changes although some of those which occur are found more often than can be expected by chance and may therefore have some diagnostic significance. These include the MG and W chromosomes in the malignant dysproteinaemias (myelomatosis and macroglobulinaemia) and possibly the deleted E chromosome in the lymphomas.

Whilst the changes found are usually compared with the normal in diagnosis, differentiation from leukaemoid reaction is more important and more data on chromosome changes in such conditions are required.

Chromosome studies have proved of little value in treatment; although it is theoretically desirable to rid the body of all abnormal cell clones this has not proved practical and cytogenetic examinations have no advantage over the conventional methods in the control of drugs. A possible exception might be a change in treatment in chronic granulocytic leukaemia if "blastic change" was predicted by increased aneuploidy when this was not evident in the gross morphology; more studies are required to settle this point.

Cytogenetic studies have a role in prognosis, by aiding a correct diagnosis, for example between chronic granulocytic leukaemia and polycythaemia vera, by predicting leukaemia in preleukaemic

patients and by forecasting clinical deterioration, for example in the blastic change of chronic granulocytic leukaemia.

The contribution of chromosome studies to an understanding of the aetiology and pathogenesis of leukaemia is substantial but no one cause has been found and much remains unknown. Perhaps the leukaemias and allied disorders represent many diseases with different aetiology and should not be lumped together. Chronic granulocytic leukaemia is unique in being the only neoplasm with a constant and characteristic chromosomal abnormality. The Ph chromosome is present in all myeloid cells (erythrocytic and platelet precursors as well as granulocytes), indicating that there is a common stem cell. The deletion of the small G group chromosome in one cell may well be the primary factor in the leukaemic process. There is good evidence that this change is acquired and it seems likely that if it does not initiate the process, at least it is related to the pathogenesis of the disorder and is less likely to be secondary. The same cannot be said with equal assurance about the other leukaemias, lymphomas and allied disorders where the significance of chromosomal changes is not always clear. Visible chromosomal abnormalities cannot be found in some cases and cannot therefore be an aetiological factor in every patient; where they do occur they may be responsible for the cell becoming malignant, might be concerned in progression of neoplasm or may be important secondary changes. In favour of primary involvement is the constant change in an individual patient and in some cases the individual condition, such as the W chromosome in macroglobulinaemia, and in the association of leukaemias with conditions characterized by other structural and chromosomal abnormalities, such as Down's and Fanconi's syndromes. Chromosomal abnormalities may be necessary for the progression of disorder in some patients, even if they are not the primary stimulus, by giving rise to altered cell metabolism with a growth advantage. Alternatively, as they cannot be found in all cases the chromosome changes may be only secondary and play no part in pathogenesis. It may be, however, that they are necessary in some instances and not in others, depending on the genetic make up of the cell. This is supported by the evidence from twins concordant for acute leukaemia in which monozygotic pairs were found to have several similar abnormalities, whereas dizygotic twins were dissimilar.

Chromosomal abnormalities have been produced by a variety of agents, including viruses, ionising radiation, chemicals and

drugs. These changes, though not the same, bear some resemblance to the changes which occur in some of the leukaemic states, but as yet no definite progression from these changes to the leukaemic state has been observed. It seems reasonable, however, to suppose that they are implicated either directly or by making the cell more susceptible to stimulation by another leukaemic agent. Possibly, multiple factors are involved and visible chromosomal abnormalities may be one stage in a stepwise progression towards malignancy.

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ADDENDUM

Since completing the manuscript some further papers have been reviewed and are listed below together with, in some instances, appropriate comments.

BENVENISTI, D. S., and ULTMANN, J. E. (1969). Eosinophilic Leukaemia. Report of five cases and review of literature. *Annals of Internal Medicine*, **71,** 731–745.
Chromosomes were studied in two of their cases and were normal.

BRAUN-FALCO, O., and MARHGESCU, S. (1969). Bloom-syndrome. A disease with Relatively High Leukaemia Morbidity. *Münchener Medizinische Wochenschrift*, **111,** 65–69.
A review of twenty-four cases in the literature. Cytogenetic studies showed anomalies in patients with the syndrome and in healthy members of their families. The anomalies are similar to those found in patients with leukaemia; this, and the fact that so far three patients died of acute leukaemia, indicates a poor prognosis.

British Journal of Haematology (1970). Instructions to Authors. **18,** 3–12.
This article contains a useful list of abbreviations, symbols and conventions which will be widely used by British Haematologists.

BUCHANAN, J. G., and BECROFT, D. M. O. (1970). Down's Syndrome and Acute Leukaemia: A Cytogenetic Study. *Journal of Medical Genetics*, **7,** 57–69.
The bone marrow cells from a three-year-old girl with Down's syndrome and Acute Myeloblastic Leukaemia contained two cell lines, one showed Trisomy 21 only, the other presumed to be a leukaemic clone had a consistent chromosome number of 49 and a karyotype 49, XX, C+G+G+.

COHEN, M. M., HIRSCHHORN, K., and FROSCH, W. A. (1969). Cytogenetic Effects of Tranquilizing Drugs *in vivo* and *in vitro*. *The Journal of the American Medical Association*, **207,** 2425 and 2426.
Suitable doses of chlorpromazine *in vitro* and a variety of phenothiazines *in vivo* did not cause chromosome damage.

DE LA CHAPELLE, A., WENNSTROM, J., WASASRJERNA, C., KNUTAR, F., STENMAN, U-H., and WEBER, T. H. (1970). Apparent C Trisomy in Bone Marrow Cells: Report of two cases. *Scandinavian Journal of Haematology*, **7,** 112–122.
One patient suffering from an atypical myeloproliferative syndrome had a supernumary C group chromosome in all his dividing bone marrow cells for over a year. The other patient with acute myelomonocytic leukaemia had a mixture of normal and C trisomic cells in his bone marrow.

DOSIK, H., HSU, L. Y., TODARO, G. J., LEE, S. L., HIRSCHHORN, K., SELIRIO, E. S., and ALTER, A. A. (1970) Leukaemia in Fanconi's Anaemia: Cytogenetic and Tumor Virus Susceptibility Studies. *Blood*, **36,** 341–352.
A family with two affected male children one of whom developed acute myelomonocytic leukaemia is described. Chromosome preparations in both parents showed abnormalities.

EZDINLI, E. Z., SOKAI, J. E., AUNGST, C. W., KIM, U., and SANDBERG, A. A. (1969). Myeloid Leukaemia in Hodgkin's Disease. Chromosomal Abnormalities. *Annals of Internal Medicine*, **71,** 1097–1104.
Chromosomal analysis of bone marrow cells in three cases of myeloid leukaemia occurring among a population of patients with Hodgkin's disease showed abnormalities similar to those usually described in the respective types of leukaemia. There were no chromosomal abnormalities in the bone marrow cells from 28 patients with Hodgkin's disease without leukaemia.

FORNI A., and MOREO, L. (1969). Chromosome Studies in a Case of Benzene-Induced Erythroleukaemia. *European Journal of Cancer*, **5.** 459–463.
A pseudo-diploid stem line 46E—M++ was present, in the bone marrow and peripheral blood.

GIANNELLI, F. (1970). *Human Chromosome DNA Synthesis* (Karger Monographs in Human Genetics, 5). Basel: S. Karger Ag.
Chromosomes 19 and 20 can now be separately identified by combined measurement and autoradiographic studies.

HARTWICH, G., SCHWANITZ, G., and BECKER, J. (1969) Chromosome Anomalies in a Case of Benzene Leukaemia. *German Medical Monthly*, **14,** 449–450.
This is an English translation of the article given in the references under Hartwich *et al.* (1969).

HILTON, H. B., LEWIS, I. C., and TROWELL, H. R. (1970) C Group Trisomy in Identical Twins with Acute Leukaemia. *Blood*, **35,** 222–226.
This paper is referred to on page 113 as a personal communication.

HIRSCHMAN, R. J., SHULMAN, N. R., ABUELO, J. G., and WHANG-PENG. J. (1969). Chromosomal aberrations in two cases of inherited aplastic anaemia with unusual clinical features. *Annals of Internal Medicine*, **71,** 107–117.
Cultured peripheral lymphocytes of both patients showed a high prevalence of chromosomal breaks characteristic of Fanconi's anaemia.

HOWLETT, R. M. (1970). *A Study of Human Chromosome Lengths associated with Autoradiographic Patterns.* (Ph.D. Thesis, London University).
The work referred to under Giannelli (1970) above is detailed in this thesis.

ISHIHARA, T., and KUMATORI, T. (1969). Cytogenetic Studies on Fishermen exposed to Fallout Radiation in 1954. *Japanese Journal of Genetics*, **44,** 242–251.
Stable deletions of the G chromosomes occurred more frequently than expected by chance in the cultured lymphocytes from fishermen exposed to radiation fallout at Bikini in 1954. The morphology varies from very similar to the Ph chromosome and deletions of the short arms to extremely small minutes. The presence of cells with a deleted chromosome was also observed in the bone marrow cells of one subject.

JUBERG, R. C., and JONES, B. (1970). The Christchurch Chromosome (Gp-). Mongolism, Erythroleukemia and an Inherited Gp- Chromosome (Christchurch). *New England Journal of Medicine*, **282,** 192–297.

KAMADA, N., TSUCHIMOTO, T., and UCHINO, H. (1970). Smaller G Chromosomes in the Bone-marrow Cells of Heavily Irradiated Atomic-Bomb Survivors. *Lancet*, **2,** 880–881.

KROGH JENSEN, M. (1969). Chromosome Studies in Acute Leukaemia. *Danish Medical Bulletin*, **16,** 289–293.

KROLL, W., and SCHLESINGER, K. (1970). Chromosome Studies in an infant with Acute Erythremic Myelosis. *Blood*, **35,** 282–285.
Cells from the bone marrow of a two-year-old boy with Erythremic Myelosis contained two marker chromosomes, monosomy of a G chromosome was present in half the cells studied.

LAMPERT, F. (1969) Acute Lymphoblastic Leukaemia in Siblings with Progressive Cerebellar Ataxia (Louis-Bar Syndrome). *Deutsche Medizinische Wochenschrift*, **94,** 217–220.

Lancet (1970). Single-Cell Origin for Burkitt's Tumour (Leading Article). **1,** 400
The evidence that Burkitt's tumour may be derived from a single cell is reviewed.

LAWLER, S. D., and SANGER, R. (1970). Xg Blood-groups and Clonal-origin Theory of Chronic Myeloid Leukaemia. *Lancet*, **1,** 584–585.
The Xg groups of 48 female patients with chronic myeloid leukaemia, all having the Philadelphia chromosome, were those expected of females, and differed significantly from a male distribution. This can be taken to mean either that the X*g* locus, when on a presumably normal X, is not subject to inactivation or that the theory that all the Ph^1—positive cells represent a single clone is incorrect. If the clonal theory is correct X*g* is not inactivated: if X*g* is inactivated the clonal theory is incorrect.

LISKER, R., and COBO, A. (1970). Chromosome Breakage in Ataxia—Telangiectasia. *Lancet*, **1,** 618.
An eight-year-old girl with ataxia-telangiectasia had chromosome abnormalities in bone marrow cells as well as in PHA stimulated lymphocytes.

MASTRANGELO, R., ZUELZER, W. W., ECKLUND, P. S., and THOMPSON, R. I. (1970). Chromosomes in the Spinal Fluid: Evidence for Metastatic Origin of Meningeal Leukaemia. *Blood*, **35,** 227–235.

MILES, C. P. (1970). Labeling and other effects of Antinomycin D on Human Chromosomes. *Proceedings of the National Academy of Sciences*, **65,** 585–592.

MODAN, B., PADEH, B., KALLNER, H., AKSTEIN, E., MEYTES, D., CZERNIAK, P. RAMOT, B., PINKHAS, J., and MODAN, M. (1970). Chromosomal Aberrations in Polycythemia Vera. *Blood*, **35,** 28–38.

NICHOLS, W. W., NORDEN, A., BRADT, C., BERG, B., and PELUSE, M. (1970). Cytogenetic Studies in a Case of Erythroleukaemia. *Scandinavian Journal of Haematology*, **7,** 32–36.
No abnormalities were seen.

NIELSON, V. G., and KROGH JENSEN, M. (1970). Pernicious Anaemia and Acute Leukaemia. *Scandinavian Journal of Haematology*, **7,** 26–31.
Although chromosomal abnormalities can be found in pernicious anaemia there is no evidence that the association between pernicious anaemia and acute leukaemia is other than a chance one.

ONESTI, P., and WOODLIFF, H. J. (1970). The Philadelphia Chromosome in a patient with Acute Leukaemia. *The Medical Journal of Australia*, **1,** 544–546.

O'RIORDAN, M. L., BERRY, E. W., TOUGH, I. M. (1970). Chromosome Studies on Bone Marrow from a Male Control Population. *British Journal of Haematology*, **19,** 83–90.

PRIETO, F., EGOZCUE, J., FORTEZA, G., and MARCO, F. (1970) Identification of the Philadelphia (Ph–1) Chromosome. *Blood*, **35,** 23–27.
Evidence is presented that the Philadelphia chromosome in the case studied was derived from one of the two early replicating Gs which the authors consider to be G22s in distinction to the late replicating Gs (G21s) which are trisomic in Down's Syndrome.

PROPP, S., and LIZZI, F. A. (1970). Philadelphia Chromosome in Acute Lymphocytic Leukaemia. *Blood*, 36, 353–360.

SCHNEIDER, G. J., CHONE, B., *et al.* (1969). Chromosomal Aberrations in a Radiation Accident. Dosimetric and Haematologic Aspects. *Radiation Research*, **40,** 613–617.

SINKOVICS, J. G., DREWINKO, B., and THORNELL, E. (1970). Immunoresistant Tetraploid Lymphoma Cells. *Lancet*, **1,** 139–140.

SNYDER, A. L., LI, F. P., HENDERSON, E. S., and TODARO, G. J. (1970). Possible Inherited Leukaemogenic Factors in Familial Acute Myelogenous Leukaemia. *Lancet*, **1**, 586–589.

TSUCHOMOTO, T., ISHII, Y., USHINO, H., and INOUE, S. (1970). Paroxysmal Nocturnal Haemoglobulinuria with Chromosome abnormalities; Possible Preleukaemia. *Lancet*, **1**, 617–618.

VISFELDT, J., FRANZEN, S., and TRIBUKAIT, B. (1970). Cytogenetic Studies in Myeloproliferative Syndrome and some atypical myeloid disorders. Preliminary Communications. *Acta pathologica et microbiologica Scandinavica*, Section A: Pathology. **78**, 80–84,
Three of six patients whose bone marrow contained abnormal clones following the treatment of Polycythemia Vera for a number of years died within six months after the chromosomal analyses.

WERTELECKI, W., and SHAPIRO, J. R. (1970). 45, XO Turner's Syndrome and Leukaemia. *Lancet*, **1**, 789–790.

WHANG-PENG, J., LEVENTHAL, B. G., ADAMSON, J. W., and PERRY, S. (1969). The Effect of Daunomycin on Human Cells *in vivo* and *in vitro*. *Cancer*, **23**, 113–121.

INDEX

INDEX

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