PROGRESS IN

Medical Genetics

VOLUME IX

PROGRESS IN

Medical Genetics

Volume IX

Edited by

ARTHUR G. STEINBERG, Ph.D.

Francis Hobart Herrick Professor of Biology, Department of Biology, and Professor of Human Genetics, Department of Reproductive Biology, Case Western Reserve University, Cleveland, Ohio

and

ALEXANDER G. BEARN, M.D.

Professor and Chairman of the Department of Medicine, Cornell University Medical College, and Physician-in-Chief, The New York Hospital, New York

GRUNE & STRATTON

New York and London

1973

Grune & Stratton, Inc., 111 Fifth Avenue, New York, New York 10003

Library of Congress Catalog Card Number 60-53514
International Standard Book Number 0-8089-0781-6
Printed in the United States of America

Foreword

The splendid contributions in this volume clearly demonstrate that the title of this series, *Progress in Medical Genetics,* is more felicitous than was realized when it was selected.

A year or so ago, the drama of human chromosome analysis seemed to have come to an end. Indeed, about three years ago a prominent geneticist said that human chromosome analysis had become a form of routine taxonomy. Now the development of new staining techniques has revitalized the field and opened new vistas for human cytogenetics. Orlando and Dorothy Miller and Dorothy Warburton elegantly describe these exciting advances and weigh their implications (Chapter 1).

Knowledge of the early development of the human and of his genetic control is essential for many aspects of medical care, e. g., understanding of congenital anomalies, fertility control (including enhancement), the causes of twinning, and so on. The development of techniques for in vitro fertilization of the human egg and for maintaining the zygote in vitro for a period of time has provided opportunities for analyses which could not previously be done. These intriguing developments are described by Ruth E. Fowler and R. G. Edwards in Chapter 2.

Alfred G. Knudson, Jr., Louise C. Strong, and David E. Anderson describe, in Chapter 3, the recent development of a new analytical approach to the genetics of cancer in man. Their ingenious approach promises to remove much of the haziness from this very difficult genetic problem. We are pleased to be able to present this clear and exciting review of new developments by the people responsible for them.

The ultimate goal of human genetics is its application in the care of patients. Medical geneticists are becoming increasingly aware that experimentation in methods of application is essential if care of patients with genetic diseases is not to tax the supply of trained personnel (medical and paramedical) and of finances. Outstanding work in this area has been carried on in Montreal for several years. We have been fortunate in being able to persuade pioneers Carol L. Clow, F. Clarke Fraser, Claude Laberge, and Charles R. Scriver to describe their work for our readers (Chapter 4).

Geneticists whose training was completed before 1959 were startled to learn that XXY mammals are male and that XO mammals are female (in *Drosophila* they are female and male, respectively). Since that initial finding, knowledge concerning genetic control of sexual difference in terms of gene action has

progressed at an accelerating rate. Daniel D. Federman, who is responsible for a large portion of these advances, describes them in Chapter 5.

The association of hypercholesterolemia with coronary artery disease and the clear familial correlation of plasma cholesterol levels have stimulated much research on the genetics of this and other plasma lipids and on the diseases associated with their abnormal levels. Robert S. Lees, Dana E. Wilson, Gustav Schonfeld, and Shelley Fleet, workers at Massachusetts Institute of Technology, have been major contributors to our clinical, biochemical, and genetic knowledge of these dyslipoproteinemias. They describe them and evaluate our current knowledge about them with inspiring clarity and insight (Chapter 6).

Earlier in this foreword we remarked on the rapid advances being made in medical genetics. This is dramatically revealed by a comparison of the fine review of pharmacogenetics by Arno G. Motulsky in Volume III with the equally fine and exciting review of the same subject by Elliot S. Vesell in Chapter 7 of this volume.

Once again, we take the opportunity to pay our awe-inspired respects and gratitude to the authors for their splendid articles. We are humbled by their splendor.

We are most pleased to acknowledge the assistance of Dr. Judith R. Tennant as Editorial Consultant.

A.G.S.
A.G.B.

Contents

FOREWORD v

1. APPLICATION OF NEW STAINING TECHNIQUES TO THE STUDY OF HUMAN CHROMOSOMES
Orlando J. Miller, Dorothy A. Miller, and Dorothy Warburton 1

2. THE GENETICS OF EARLY HUMAN DEVELOPMENT
Ruth E. Fowler and R. G. Edwards 49

3. HEREDITY AND CANCER IN MAN
Alfred G. Knudson, Jr., Louise C. Strong, and David E. Anderson 113

4. ON THE APPLICATION OF KNOWLEDGE TO THE PATIENT WITH GENETIC DISEASE
Carol L. Clow, F. Clarke Fraser, Claude Laberge, and Charles R. Scriver 159

5. GENETIC CONTROL OF SEXUAL DIFFERENCE
Daniel D. Federman 215

6. THE FAMILIAL DYSLIPOPROTEINEMIAS
Robert S. Lees, Dana E. Wilson, Gustav Schonfeld, and Shelley Fleet 237

7. ADVANCES IN PHARMACOGENETICS *Elliot S. Vesell* 291

AUTHOR INDEX 369

SUBJECT INDEX 379

CHAPTER 1

Application of New Staining Techniques to the Study of Human Chromosomes

Orlando J. Miller

Dorothy A. Miller

Dorothy Warburton

Departments of Human Genetics and Development and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, New York.

THE QUINACRINE AND QUINACRINE MUSTARD FLUORESCENCE TECHNIQUES 2
Mechanism of Action 2
Binding 2
Fluorescence 5
Method 5
The Human Karyotype 6
Normal chromosomes 6
Common variants 7
Chromosomal Abnormalities 9
Autosomal abnormalities 9
Sex chromosome abnormalities 12
Y Bodies in Interphase Nuclei 14
Meiotic Chromosomes 16
Tumors and Cell Lines 17
Studies in Other Species 18
Interspecific Somatic Cell Hybrids 18
Identification of specific human chromosomes 18
Application to Cytogenetic Mapping 18
Evaluation of Quinacrine Method 19
Other Fluorochromes 19
MODIFIED GIEMSA STAINING METHODS 20
Characteristics of Cationic Dyes 20

This work was supported in part by grants from the National Foundation-March of Dimes, and the U.S. Public Health Service: The National Institute of General Medical Sciences and the National Cancer Institute (GM 18153; CA 12504).

Centromeric Heterochromatin Staining (C Banding) 21
Giemsa Banding Techniques (G Banding) 23
Acetic-saline-Giemsa (ASG) 23
Denaturation-renaturation 24
Giemsa 9 24
Reverse Giemsa banding 24
Proteolytic enzyme treatment 25
FLUORESCENT ANTIBODY TECHNIQUE 27
RELATED CHARACTERISTICS 28
Time of DNA Replication 28
Secondary Constrictions 30
COMPARISON OF CHROMOSOME BANDING TECHNIQUES 30
MECHANISMS OF CHROMOSOME BANDING 34
Role of DNA 35
Base composition 35
Native vs. denatured DNA 36
Role of Protein 37

The past two years have witnessed a revolution in our understanding of the human karyotype. This has been the result of the development of staining techniques which produce chromosome banding so characteristic that every human chromosome can now be identified. The first technique, which was worked out by Caspersson and his associates, involves fluorescence microscopy of quinacrine or quinacrine mustard-stained cells. A flood of new methods has followed, and some of these are so simple and easy to perform that one of them may, in time, replace the fluorescent banding technique. At the present time, however, only the latter method has been proven to give consistent and reliable results, and its applications already cover the entire range of human cytogenetics. The quinacrine fluorescent technique will therefore be described first, with examples of its application in various areas. Other new staining methods will then be described, and their present and potential uses discussed. A continuing development of still newer techniques can confidently be predicted as the implications of the applicability of still other advances in molecular biology become clear.

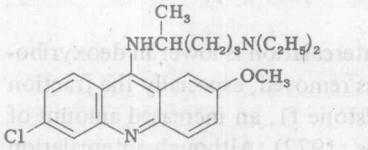
THE QUINACRINE AND QUINACRINE MUSTARD FLUORESCENCE TECHNIQUES

Mechanism of Action

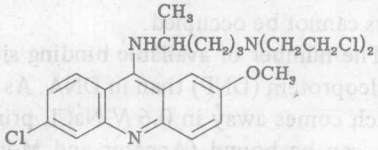
Binding. Quinacrine (Atebrin) is an acridine dye which was extensively used as an antimalarial agent during World War II, and continues to find limited use as an antiprotozoal agent. Its antimalarial action is based on its capacity to combine with deoxyribonucleic acid (DNA) and block ribonucleic acid (RNA)

synthesis (Kurnick and Radcliffe, 1962; O'Brien et al., 1966). Acridine dyes such as quinacrine (Q) and quinacrine mustard (QM, Fig. 1) combine much more strongly with native DNA than with depolymerized DNA (Morthland et al., 1954). The binding is independent of pH in the range from 3.7-7.4 (Peacocke and Skerrett, 1956).

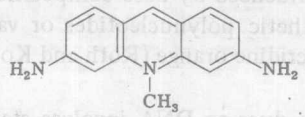
Acridine and phenanthridium (e.g., ethidium bromide, Fig. 1) dyes combine with DNA by at least two processes (Blake and Peacocke, 1968). The first is by intercalation (Lerman, 1963) of the flat triple-ring systems between adjacent



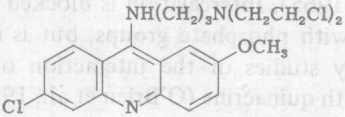
Quinacrine



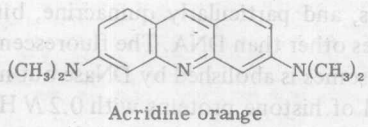
Quinacrine mustard



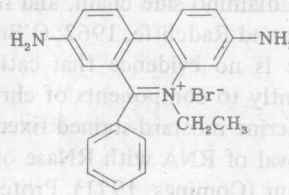
Acriflavine



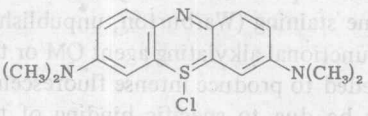
Propyl quinacrine mustard



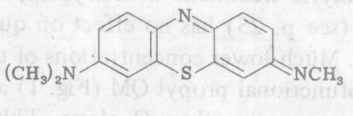
Acridine orange



Ethidium bromide



Methylene blue



Azure B

FIG. 1.–Some basic dyes which bind to DNA.

base pairs in the DNA double helix with one of the active basic groups contiguous to a free phosphate radical (Michaelis, 1947). This makes the DNA molecule longer, as demonstrated autoradiographically by Cairns (1962), who showed that T2 phage DNA molecules were as much as 50% longer in the presence of the acridine dye proflavine than in its absence. The maximum amount of acridine dye which can be bound by intercalation, 0.44 molecules per base pair (Peacocke and Skerrett, 1956) (1 quinacrine molecule per 3.8 nucleotides, according to Kurnick and Radcliffe, 1962) is just about the amount expected if intercalation occurs at random and adjacent spaces between base pairs cannot be occupied.

The number of available binding sites for intercalation is lower in deoxyribonucleoprotein (DNP) than in DNA. As protein is removed, especially the fraction which comes away in 0.6 *N* NaCl, primarily histone f1, an increased amount of dye can be bound (Angerer and Moudrianakis, 1972). Although intercalation requires a double helical structure, heat-denatured and cooled DNA binds ethidium bromide and acridine dyes just as well as native DNA, presumably because a sufficient proportion of hydrogen-bonded regions is still present (Waring, 1965). Intercalation is blocked by divalent cations such as Mg^{2+}, which interact with phosphate groups, but is not influenced by base composition, as shown by studies of the interaction of synthetic polynucleotides or various DNAs with quinacrine (O'Brien et al., 1966), acridine orange (Roth and Kochen, 1971) or ethidium bromide (Waring, 1965).

The second method of binding of acridine dyes to DNA involves stacking additional dye molecules on the surface of the double helix by electrostatic interaction with any available negatively charged phosphate groups (Waring, 1965; LePecq and Paoletti, 1967). In the case of quinacrine, this involves the aliphatic diamino side chain, and results in a stabilization of double helical DNA (Kurnick and Radcliffe, 1962; O'Brien et al., 1966).

There is no evidence that cationic dyes, and particularly quinacrine, bind significantly to components of chromosomes other than DNA. The fluorescence of quinacrine mustard-stained fixed chromosomes is abolished by DNase but not by removal of RNA with RNase or removal of histone proteins with 0.2 *N* HCl for 1 hour (Comings, 1971). Proteolytic enzyme treatment (pronase for 1 hour at 37°) modifies the apparent binding of quinacrine by producing even fluorescence, but the basis of this effect is unclear (Comings, 1971). Milder proteolytic treatment with trypsin, sufficient to produce banding with Giemsa stain (see p. 25) has no effect on quinacrine staining (Warburton, unpublished data). Much lower concentrations of the difunctional alkylating agent QM or the monofunctional propyl QM (Fig. 1) are needed to produce intense fluorescence of chromosomes than Q alone. This may be due to specific binding of the alkylating derivatives to the N-7 position of guanine (G), which is by far the most reactive site in purified DNA, although the N-3 and N-7 positions of

adenine (A) are also reactive (Lawley, 1966). Inadequate information is available on alkylating reactions with nucleoproteins (Shapiro, 1969) to speak with assurance of the binding of QM to chromosomes. It is clear, however, that in mammalian chromosomes the fluorescent banding patterns produced with QM are virtually identical to those produced with quinacrine (Miller et al., 1971a; Pearson et al., 1971; Sumner et al. 1971a), in spite of the earlier experience of Caspersson et al. in *Trillium* (1969a).

Fluorescence. Most acridine and phenanthridium dyes are fluorescent and retain this property when bound to DNA. Quinacrine fluorescence, and presumably that of similar dyes, is dependent on the double-stranded nature of DNA (Lerman, 1963; Weisblum and de Haseth, 1972). The interaction of the dyes with DNA, or synthetic polynucleotides (Steiner and Beers, 1959), can produce significant alterations in fluorescence. The fluorescence of ethidium bromide is markedly enhanced by binding to DNA (LePecq and Paoletti, 1967). This enhancement involves only the dye that is bound by intercalation (Angerer and Moudrianakis, 1972). The opposite effect, quenching of fluorescence, is sometimes observed and appears to be related to base composition. For example, at high ionic strength, which permits dye binding only by intercalation, quenching of acridine orange fluorescence occurs with the synthetic nucleotide polyadenylic acid (polyA) but not with polycytidylic acid (polyC) (Steiner and Beers, 1961). DNA rich in adenine and thymine (A+T) has been reported to enhance acriflavine, proflavine, or quinacrine fluorescence, whereas DNA rich in guanine and cytosine (G+C) quenches the fluorescence (Tubbs et al., 1964; Thomes et al., 1969; Weisblum and de Haseth, 1972). The intensely fluorescent quinacrine-stained region of the Y chromosome in an insect of the family Drosophiladae, *Samoaia leonensis,* appears to be composed almost solely of A and T bases (Ellison and Barr, 1972).

Many of the usual fixatives, including 95% ethanol, osmic acid, and 10% Formalin, are said to produce quenching of fluorescence, and freeze-drying may thus provide brighter fluorescence (Bruyn et al., 1950). Acridine dye fluorescence is quenched in basic solution; so these dyes should be used at an acid pH (Metcalf and Patton, 1944; Steiner and Beers, 1959).

Method

Either quinacrine or one of its alkylating derivatives, such as quinacrine mustard, can be used to produce chromosome banding. Metaphase preparations fixed in 3:1 methanol:acetic acid are stained in a 0.5% solution of quinacrine or a 0.005% solution of QM for 5-10 min, washed in tap water, and wet mounted. Good results have been obtained with a variety of buffers or even water as a mounting medium. Fluorescence microscopy is carried out using an HBO 200 W high-pressure mercury lamp with a BG-12 excitor filter and a 530 nm barrier

filter. Because of fading of the fluorescence, as well as to facilitate comparison among chromosomes and cells, it is important to have a photographic record of each cell. The requirements for the most suitable photography are clearly described by Breg (1972).

Chromosomes can be recognized by the consistent pattern of brightly and dully fluorescing bands, which are identical in homologs. For best visualization of the bands, the chromosomes should be extended with chromatids lying closely together. In more contracted chromosomes several bright bands may be seen as one single bright area, although the over-all pattern may still allow chromosome identification.

The Human Karyotype

Normal Chromosomes. Caspersson and his associates (1970g) arranged the fluorescent human chromosomes into a karyotype in which the number assigned to each chromosome corresponded, where possible, to the number which had been assigned previously by other methods of identification. This karyotype has been accepted as the international standard (Paris Conference, 1971) (Fig. 2). The chromosome pairs were arranged first by morphological characteristics (primarily length) and centromeric index. Chromosomes 4, 5, 13, 14, 15, 17, 18,

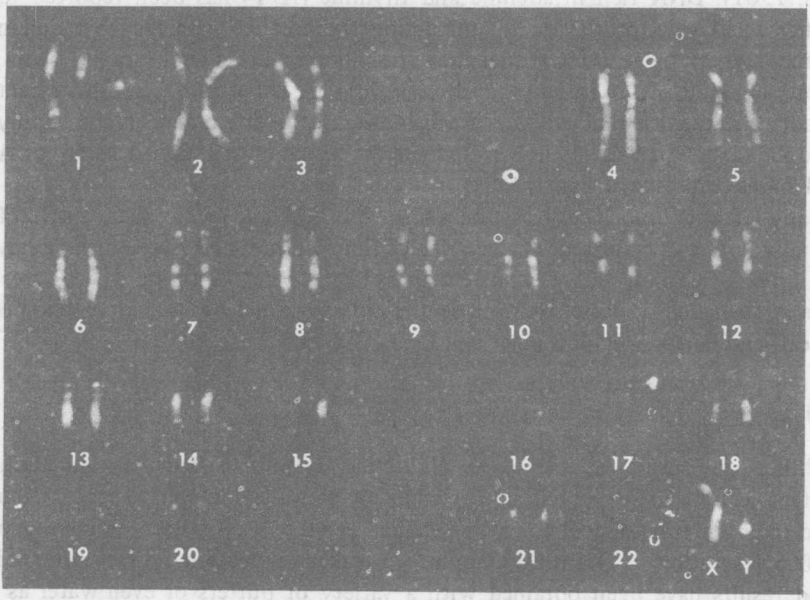


FIG. 2.–Karyotype of a metaphase cell from a male, stained with quinacrine. Note the characteristic banding patterns of each chromosome pair. From Breg, 1972. (By permission of Williams & Wilkins Co.)

and the X were given numbers which corresponded to those assigned on the basis of terminal DNA replication studies. Chromosomes 1, 9, and 16 were oriented so that the secondary constriction was in the long arm. (Some early karyotypes show chromosomes 1 with the long arm at the top.) Since insufficient criteria existed for distinguishing between chromosomes 10 and 12 or between 19 and 20, these numbers were assigned arbitrarily. For historical reasons the chromosome which is trisomic in Down's syndrome (trisomy 21 syndrome) continues to be called number 21, although number 22 is slightly longer (Hamerton, 1971). A brief description of the banding pattern of each chromosome pair has been presented by Caspersson et al. (1971g).

In addition to visual methods, Caspersson and his associates (1971f) have utilized photometric methods involving the production of densitometric tracings from photographic negatives or prints for analyzing the intensity of fluorescence along chromosomes (Fig. 3) and have used Fourier analysis by computer as a means of synthesizing the resultant patterns. In this way, the pattern type of each C-group chromosome has been delineated and differentiated from that of every other C-group chromosome. Thus, accurate automatic machine recognition and classification of human chromosomes is now possible and can be expected to become available to many workers before very long. Television techniques can also be used, instead of the photographic method of recording fluorescence patterns, with resultant speed-up of large-scale classification work (Caspersson et al., 1970c). In the meantime, visual analysis of fluorescent banding patterns provides a very adequate means of chromosome identification.

Common Variants. Some of the chromosomes have been found to have variant forms in the normal population. These variants are usually marked by a different size or intensity of fluorescence of a specific band. It is assumed that they are inherited in simple Mendelian fashion and could be used in genetic marker studies. For example, Caspersson et al. (1970h) reported that a mother and son each had a D-group chromosome with very bright fluorescence in the short-arm region. Analysis of the variants is complicated by the fact that the degree of brightness or the size of the very bright region appears to be a continuous variable in the population, though not in the individual.

An intensely bright fluorescent band may be present in the pericentric region of number 3 (Fig. 2). Schnedl (1971b) reported finding such a bright region of the number 3 chromosomes in 59% of 50 individuals, including those who had two, one, or no bright band. The long arm of chromosome 4 sometimes has a bright band adjacent to the centromere. This can be an aid in distinguishing chromosome 4 from 5. Each of the acrocentric chromosomes may have bright satellites or short arms. The presence of a bright centromeric region on number 13 is a particularly common variant (Fig. 2). The variant forms of the other D- and G-group chromosomes, e.g., chromosome 14 in Fig. 11, are less common and may be more useful as somatic markers. Examples of each of these may be found in Evans et al. (1971).

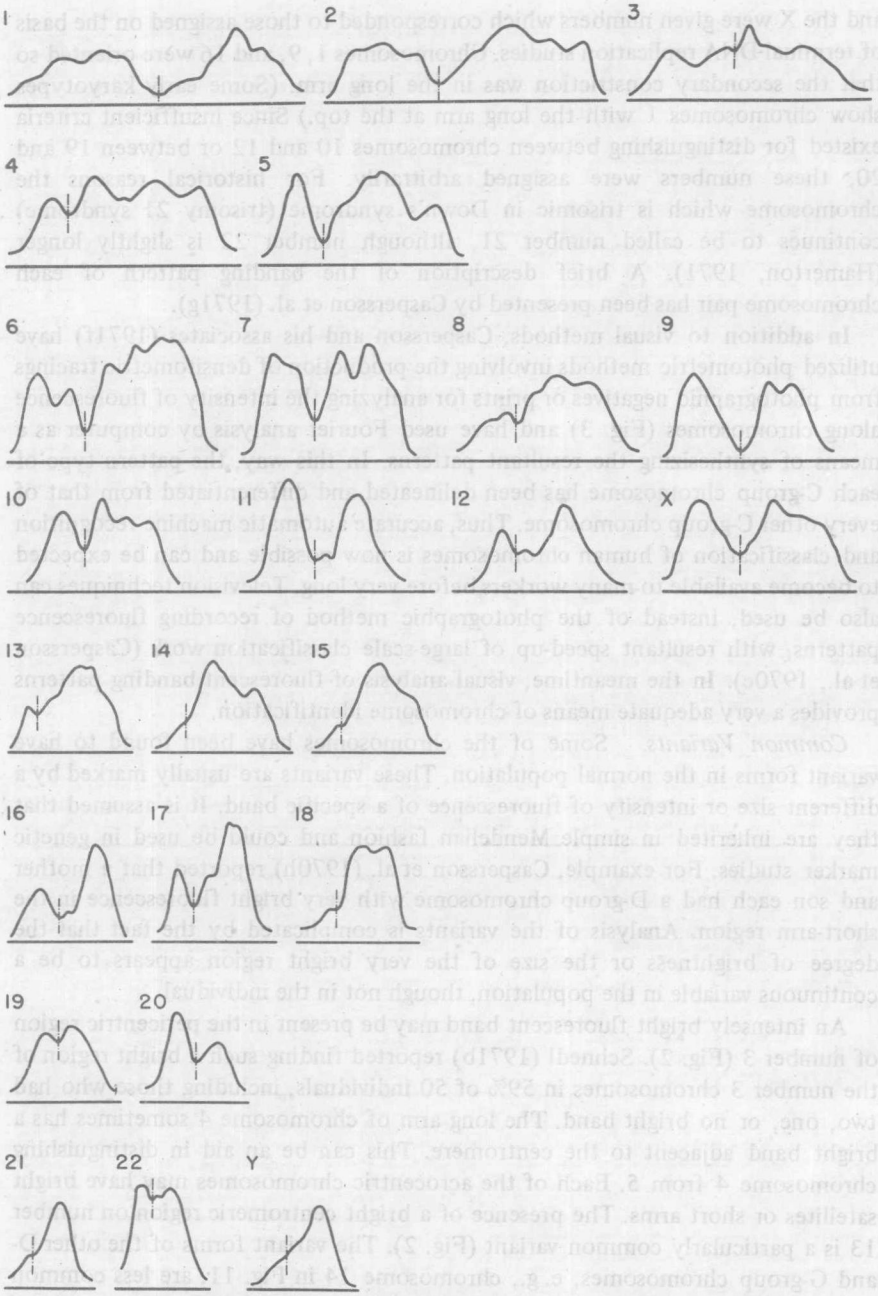


FIG. 3.–Photometrically determined quinacrine mustard fluorescence patterns for the 24 types of human metaphase chromosomes. (From Caspersson et al., 1971g.)

The variant forms of the Y chromosome have received considerable attention. Caspersson et al. (1970h) reported that the length of the intensely fluorescent material of the Y chromosome in a given male was constant. There have been reports of very small Y chromosomes which had little or no intensely fluorescent region (Borgaonkar and Hollander, 1971; Lewin and Copen, 1971; Robinson and Buckton, 1971; Wahlström, 1971). Very long Y's which had a long region of intense fluorescence have also been reported (Knuutila and Gripenberg, 1972; Robinson and Buckton, 1971; Wahlström, 1971; Wilson et al., 1971). Bobrow et al. (1971) and Schnedl (1971c) measured the dully and intensely fluorescent segments of Y chromosomes from normal males. The length of the very bright, but not of the dull, region varied widely; this variation accounted for almost the entire difference in total length of the Y chromosomes.

Additional intensely fluorescent material in the Y chromosome may be present as several bands. Kim et al. (1971) noted the presence of two brightly fluorescent bands in the long arm of the Y chromosome in 5 of 21 men studied. Walhström (1971) reported finding a father and son both of whom had very long Y chromosomes with four intensely fluorescent bands.

Chromosomal Abnormalities

Autosomal Abnormalities. i. Trisomy. The identity of an additional chromosome can be determined easily by the quinacrine fluorescence method because the entire banding pattern of the chromosome can be observed. Down's syndrome has been extensively studied (Caspersson et al., 1970b; Alfi et al., 1971; Breg et al., 1971b; Mikkelsen, 1971; O'Riordan et al., 1971; Schwinger et al., 1971b). In each case the extra G-group chromosome was a number 21, the shorter chromosome with a bright band in the long arm. Down's syndrome (trisomy 21 syndrome), therefore, invariably involves chromosome 21 and never chromosome 22. In some cases a variant number 21 with a bright satellite has been present in duplicate. The presence of such markers made it possible to determine that the additional chromosome was of maternal origin in one case (Licznerski, and Lindsten, 1972).

A few other types of autosomal trisomy have been investigated. In a case with C trisomy/normal mosaicism, the extra chromosome was identified as a number 8 by its fluorescence pattern (Grouchy et al., 1971). Caspersson et al. (1972b) studied 4 cases of trisomy of this same chromosome.

The D-group chromosomes in 2 patients with the 13-trisomy syndrome included three chromosomes with the fluorescent banding pattern expected for number 13. These chromosomes also had the autoradiographic terminal labeling pattern of chromosome 13 (Miller et al., 1971a). Two apparent D trisomies were shown by fluoresence analysis to have partial (tertiary) trisomy of the long arm of chromosome 14 as a result of nondisjunction in a parent heterozygous for a reciprocal translocation (Allderdice et al., 1971; Miller et al., 1971a; Breg et al., 1972a).

ii. Centric fusion translocations. Identification of chromosome segments translocated to other chromosomes has been possible because the translocated segments retain the banding pattern characteristic of the same segments in the chromosome of origin. Translocations which involve entire chromosome arms are relatively easy to identify, and the translocations which have been studied most extensively by the fluorescence technique are those of the centric fusion types: D/D D/G, and G/G. A wide variety of combinations has been demonstrated: 13/13 (Miller et al., 1971a), 13/14 (Caspersson et al., 1971d; Miller et al., 1971a), 14/14 (Caspersson et al., 1971d), 13/21 (Caspersson et al., 1971d; O'Riordan et al., 1971), 14/21 (Alfi et al, 1971; Caspersson et al., 1971d; Miller et al., 1971a; O'Riordan et al., 1971), 15/21 (Alfi et al., 1971), 21/21 (Alfi et al., 1971; Breg et al., 1971b; Caspersson et al., 1971d; O'Riordan et al., 1971), and 21/22 (Caspersson et al., 1971d; Mikkelsen, 1971). In two translocations, previous identification by autoradiography was found to be in error. A 14/21 had been misidentified as a 15/21 (Miller et al., 1971a) and a 13/21 misidentified as a 14/21 (Caspersson et al., 1971d).

iii. Reciprocal translocations. Translocations which involve smaller segments of chromosomes are difficult to analyze unless both translocation chromosomes are available for study. If both products are present very small exchanges can be identified, such as the addition of the final band of the tip of the long arm of chromosome 1 to the short arm of chromosome 18 (Breg et al., 1972b). Reciprocal translocations rarely involve whole arms of biarmed chromosomes, although this appeared to be the case in 2 patients studied by Breg et al. (1972b). In one the short arms of chromosomes 2 and 6, and in the other short arms of chromosomes 11 and 17, were exchanged, as shown by fluorescence studies. Breg et al. (1972b) have pointed out that even some apparently cytologically balanced translocations may produce an abnormal phenotype, perhaps as a result of a position effect, although a small deletion cannot be ruled out. Francke (1972) has studied a series of translocations, and has discussed whether these translocations are indeed reciprocal. However, it appears unlikely that the present techniques can be used to settle this question, because of their limited resolution.

Breg et al. (1972a) have reported on abnormalities of individual chromosomes in the C group; this is of particular interest since these chromosomes were previously identified only by group. Abnormalities were found for each chromosome in the group with the exception of number 12. One of these translocations involved three chromosomes: 6, 14, and 20 (Allderdice et al., 1971) (Fig. 4). A 6q– chromosome from which half of the long arm had been deleted and a 14/6 translocation chromosome were undetected in regular Giemsa preparations of this woman. An extra D-group chromosome in her child was identified as a number 14 by autoradiography; the extra chromosome, which was a 14/6, did not appear abnormal by this method because the replication

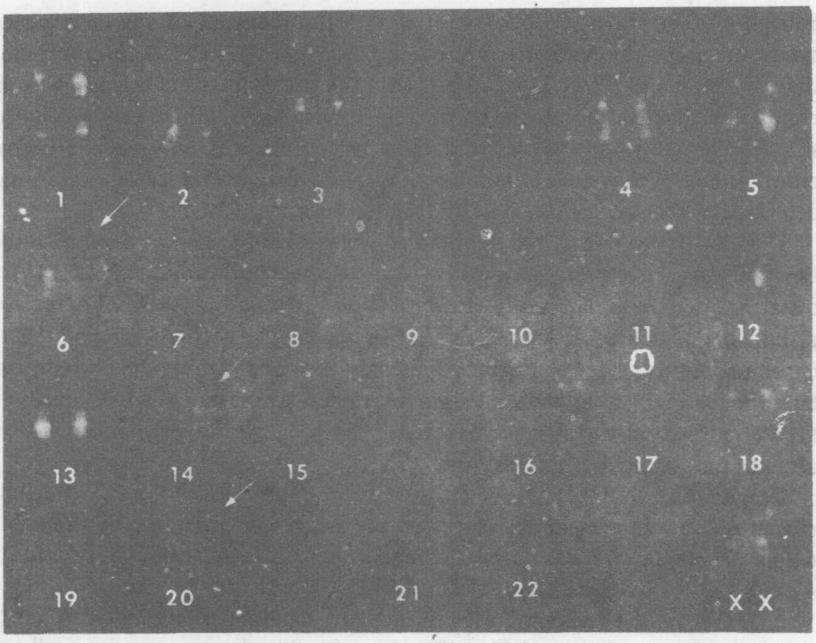


FIG. 4.–Quinacrine fluorescent karyotype of a female with three abnormal chromosomes, indicated by the arrows. The distal half of the long arm of number 14 has been translocated to the short arm of number 20, and the distal half of the long arm of number 6 has been translocated to the centromeric portion of number 14. (From Allderdice et al., 1971. By permission of Springer-Verlag, New York.)

pattern of the translocated portion of number 6 was similar to that of the 14 material it replaced.

In some cases additional methods of analysis have been used in conjunction with quinacrine fluorescence. Bobrow and Pearson (1971) showed the suspected breakpoint in a 4/18 translocation by means of densitometer tracings. The photograph of the translocation chromosome was omitted from this report, and it is unclear whether the breakpoint could be located more precisely by these tracings than by visual analysis.

Malpuech et al. (1971) reported a case in which the fluorescent pattern of a 21/18 translocation resembled that of chromosome 9. In this case Giemsa preparations proved useful: the achromatic area in the short arm of the 21 was visible in the translocation chromosome indicating that the centromere of the abnormal chromosome was derived from the 21 rather than the 18. Evans et al. (1971) illustrated the chromosomes in a t(22p+;9q–) in which the breakpoint in each chromosome was near or within a secondary constriction.

iv. Deletions. A few reports have appeared dealing with recognition of