HUMAN CHROMOSOMES:
STRUCTURE, ABNORMALITIES AND BIRTH DEFECTS

by

Ranjan Y. Goradia

Dissertation submitted to the Graduate Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Genetics

APPROVED:

Brian K. Davis
B. K. Davis, Chairman

Asuman Esen
A. Esen

R C Bates
R. C. Bates

J. O. Falkinham III
J. O. Falkinham, III

R. A. Paterson
R. A. Paterson

August, 1977

Blacksburg, Virginia

ACKNOWLEDGEMENTS

I should like to express my deep gratitude to Dr. Brian K. Davis for his guidance and support during the course of this research. We have had many lively discussions and his ever inquisitive and humorous nature has made my stay at VPI a very pleasant one.

I should like to thank Drs. Robert Bates, Asim Esen and Noel Krieg for the use of their laboratory facilities. I am also indebted to Dr. Joseph Falkinham who made many useful suggestions and Dr. Robert Paterson who provided the financial assistance needed for me to attend several conferences on birth defects.

Other special acknowledgements may be found at the end of each of the four chapters in this thesis.

I would like to express my sincere appreciation to my husband, Yogesh, without whose constant encouragement this work would not have become a reality, and to my daughter, Shree, who spent many lonely hours without 'Mom'. How shall I ever repay those precious lost hours?

Finally, I should like to thank Sandy Gay for her painstaking typing, Vincent Scott for his assistance in graphics and Bernard Baker for his help in printing the photomicrographs.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF PLATES

LIST OF PLATES (continued)

PAGE

INTRODUCTION

For most of human history, infectious diseases were the primary killers. Today, however, they are far back on the list of causes of death because of tremendous progress in biomedical research. Indeed, the diseases that are most wide spread and burdensome today are those involving disorders of the hereditary material -- the genes and the chromosomes. An estimated 15 million Americans today suffer the consequences of birth defects of varying severity. Although 20% of these cases represent the effects of agents such as infections, drugs and physical injury to fetus, and hence do not involve a heritable component, the remaining 80%, or 12 million people, carry true genetic diseases due wholly or partly to defective genes or chromosomes.(1)

These genetic defects are a major cause of infant mortality. As such, when measured in terms of the normal life expectancy they seem to claim a loss of 4.5 times as many life-years as heart diseases, 8 times as many as cancers and 10 times as many as strokes. Furthermore, it is estimated that 36% of all spontaneous abortions are caused by gross chromosomal defects, amounting to an abortion rate of more than 100,000 per year in the United States alone.(1)

In spite of these disturbing statistics, our understanding of the structure and behavior of human chromosomes is far from being complete. Indeed, it was only 20 years ago that Tijo and Levan confirmed that there are 46 chromosomes in humans and not 48 as previously thought.(2) Since the demonstration in 1959 that Down's syndrome results from an extra chromosome,(3) other syndromes have been associated with trisomies

monosomies, deletions, and translocations. The past seven years have seen an explosion of interest and progress in human cytogenetics. This has been made possible primarily by the banding techniques for chromosomal study. There is now a variety of methods which produce consistent differential staining patterns or bands on the chromosomes. It is now possible not only to identify individual chromosomes, but also to identify regions of chromosomes. With this increased resolution, chromosomal abnormalities which previously were indistinguishable from normal chromosomes can now be recognized and analyzed in detail.

In addition to the obvious applications to medical problems, the banding techniques offer insight into another area of widespread interest, chromosome structure. A eukaryotic chromosome is fibrous in nature and consists of one double stranded DNA molecule with histones, RNA and acidic proteins. The fundamental question of how these fibers are folded with a packing ratio of over 1:10,000 has been a subject of intensive research. Until a couple of years ago, the hypothesis was that several orders of chromatin coiling were involved in the packing process.[4] The chromosome configuration was believed to be the result of a primary coiling of a double DNA helix, a secondary coiling of proteins associated with the DNA, and finally, a supercoiling of the protein-associated fibers.

Evidence suggests that most of the eukaryotic chromatin is packaged into repeating arrays of globular subunits consisting of double stranded DNA associated with histones and separated by stretches of DNA not packaged into globular form. These particles are termed as nucleosomes

(nu bodies).[5,6] A model proposed by Kornberg[7] suggests that about 200 base pairs of DNA are arranged on the outside of a globular octamer of histones with the composition of $(H_4)_2(H_3)_2(H_2A)_2(H_2B)_2$. However, a later model proposed by Finch and Klug[8] is a solenoidal model for chromatin that needs histone (H_1) for stabilization of the structure.

The latest model[9] has proposed that a human chromatid is a hierarchy of helices consisting of four orders of coiling. The first order of packaging changes the DNA double helix to a string of nucleosomes, with a condensation factor of 7. The string of nucleosomes assumes a solenoid configuration in the second order of coiling, with a packing ratio increased to 40. The solenoid is folded once more to a third helix, or supersolenoid (unit fiber) thereby increasing the packing ratio to 1300-1500. It is further postulated that RNA or certain structural non-histone proteins crosslink the supersolenoid which then assumes the shape of a mitotic chromosome. We can summarize this sequence of events as follows.

	Nucleosome	Solenoid	Supersolenoid (unit fiber)	Mitotic Chromosome
Diameter	∿69 A°	300 A°	4000 A°	1.0 μm
Packing ratio	7	40	1300-1500	10,000

Unless we understand the organizational structure of the human chromosome in relation to its function, behavior and identification, the prevention of the specific birth defects that result from chromosomal abnormalities will remain incomplete.

The research presented in this dissertation consists of four

chapters that have either been published or been submitted for publication. These chapters deal with two major areas: (1) the structure of the human chromosome in relation to its function and identification (Chapters One and Two); and (2) the relationship of chromosomal abnormalities to specific birth defects (Chapters Three and Four).

A. Banding and Spiralization of Human Metaphase Chromosomes

An important tool in identifying the human chromosomes is the banding technique. Since the discovery of the Q-banding (fluorescing bands produced by acridine derivative stains) of human metaphase chromosomes by T. Casperson et al., in 1970,[10] many other techniques have been developed to study banding. One of the problems inherent in studying the structure of chromosomes is that they are fixed (acetic acid-methanol) rather than being in their native state. The second problem arises when one tries to understand the mechanism(s) that causes banding. The mechanism of G-banding (produced by using Giemsa dye as the staining agent), as concluded by Srivastava and Lucas,[11] is essentially unknown. Even though much work has been done in the technical aspect, our understanding of what causes bands in human chromosomes, why a fraction of cells do not produce banded chromosomes, and what factors are responsible for failure to obtain bands, remains partially incomplete. In contrast to banding, very little work has been done to study the spiralization of human chromosomes. Ohnuki has reported that each chromosome has a characteristic number of spirals at mitosis.[12] I have developed a new technique to produce bands, spirals and intermediate configurations depending on the temperature exposure. In this technique, I

first apply heat to fixed chromosomes and then treat them with trypsin before staining with Quinacrine or Giemsa. My interpretation is that banding is not artifactual but appears to reflect the concentrations of chromatin material along the length of the chromosome. The concentration of chromatin material in turn may be determined by chromosomal proteins. The results are presented in the first chapter.

B. Asymmetry of Sister Chromatids

In this second chapter I continue the research begun in the first chapter. My special interest here is to study the intermediate stage in which both the banding and spiralization are present. This is found to produce asymmetry in the sister chromatids, contrary to the present belief that they are identical. A possible explanation for the asymmetry is that the spirals in the sister chromatids have opposite helicities. An understanding of this asymmetry may explain the metaphase orientation of the chromatids and why each moves to the opposite pole.

C. Sex Chromosome Mosaicism in a Mother and Trisomy 13 in Her Child

The third chapter deals with a case history in which a baby with trisomy 13 was born with gross physical abnormalities and expired after 12 days. The father was found to have normal chromosomes and physical history, but the mother's physical history revealed ovarian dysfunction, lower fertility among female sibs, low finger ridge count and mosaicism for 46,XX/47,XXX in her leucocytes. The extra chromosome was a marker X, partially twisted in the short arm and slightly uncoiled in the long arm near the centromeric region. Such a combination of double trisomy

in a family -- trisomy XXX mosaicism in the mother and trisomy 13 in the child -- has never been reported before.

D. Absence of Dermal Ridges and Sex Chromosome Mosaicism

In this last chapter I study the relationship between the absence of finger prints and sex chromosomes. It is well known that chromosomal abnormalities are associated with abnormal dermatoglyphics. It has been widely observed that the autosomes have a much more drastic effect on dermatoglyphics than the sex chromosomes.[13] I present a case here in which there has been an absence of finger prints for four generations. The pedigree analysis reveals high fetal wastage. Chromosome analyses of the mother and the son show mosaicism for an extra X chromosome with a complex deletion (Xdel). This is the first time a sex chromosome has been found to give rise to complete absence of dermal ridges or ridge aplasia.

I hope the research effort presented here will bring us one step closer toward understanding birth defects.

REFERENCES

1. What are the facts about genetic diseases?, DHEW publication, NIH No. 75-370.

2. Tijo, J.H., and Levan, A.: The chromosome number of man, Hereditas (London), 42:1-6, 1956.

3. Lejeune, J., Gautier, M., and Turpin, R.; Étude chromosome somatiques de neuf infants mongoliens; C.R.Acad. Sci., Paris, 248: 1721-1722, 1959.

4. DuPraw, E.J.; DNA and Chromosomes, Holt, Rinehart and Winston, Inc., New York, 1970.

5. Olins, A.L. and Olins, D.E.; Spheroid chromatin units (nu bodies), Science, 183: 330-332, 1974.

6. Olins, A.L., Carlson, D. and Olins, D.E.; Visualization of chromatin substructure, J. of Cell Biol., 64: 528-537, 1975.

7. Kornberg, R.D.; Science, 184: 868-871, 1974.

8. Finch, J.T., and Klug, A.; Solenoidal model for superstructure in chromatin, Natl. Acad. Sci. USA, 73:(6), 1897-1901, June 1976.

9. Bak, A.L., Zeuthen, J. and Crick, F.H.C.; Higher-order structure of human mitotic chromosomes, Proc. Natl. Acad. Sci.USA, 74:(4), 1595-1599, April 1977.

10. Casperson, T., Zech, L., and Johansson, C.; Quinacrine mustard - fluorescence of human chromsomes; Exp. Cell Res. 61: 474-475, 1970.

11. Srivastava, P.K. and Lucas, F.V.; J. Genét. Hum. 24:(4), 337-350, 1976.

12. Ohnuki, Y.; Structure of chromosomes. I. Morphological studies of the spiral structure of human somatic chromosomes; Chromosoma 25: 402-428, 1968.

13. Schauman, B. and Alter, M.; Dermatoglyphics in Medical Disorders; p. 172, Springer-Verlag, New York - Heidelberg - Berlin, 1976.

CHAPTER I

BANDING AND SPIRALIZATION OF HUMAN METAPHASE CHROMOSOMES

Ranjan Y. Goradia

Brian K. Davis

The following paper has been published in Human Genetics 36: 155 - 160 (1977).

Introduction

Spiralization or coiling of human metaphase chromosomes has been produced by several different methods. The most common technique uses hypotonic treatment (Osgood et al., 1964; Ohnuki, 1965, 1966, 1967, 1968; Ruzicka, 1973). Spiralization has also been produced by hyaluronidase (Iino, 1971), low concentrations of calcium and magnesium ions (Shaw et al., 1972) and a combination of 2-mercaptoethanol, urea and SDS (Kato and Yosida, 1972).

Chromosome banding techniques have generated interest as an approach to chromosome organization (for example, Comings and Avelino, 1974), in addition to their utility in chromosome identification. It is therefore pertinent to examine the relationship between spirals and bands. Kato and Yosida (1972) compared chromosomes with Giemsa banding to those with spirals produced by hypotonic treatment. They found some correlation of frequencies and of appearance for bands and spirals. However, in contrast to band structures, the spirals were stained uniformly along the length of the chromosomes. This agrees with the measurements of Bahr et al. (1973) which show uniformity of density for hypotonic spirals. Thus, banded chromosomes are characterized by lateral uniformity and longitudinal differentiation, while spiralled chromosomes show longitudinal uniformity and lateral differentiation. The number of spirals is characteristic for each chromosome and for the stage of mitosis (Ohnuki, 1968; Ruzicka, 1973).

The purpose of this paper is to present a new method of producing spirals in human metaphase chromosomes using a combination of heat and

trypsin. This technique provides a new approach both to the mechanism of banding and to chromosome structure.

Materials and Methods

Leukocytes from normal humans were cultured in Dulbecco's modified eagle medium no. 188 g supplemented with 20% fetal calf serum, antibiotics and phytohemagglutinin. After 72-84 h growth at 37° C, colchicine (0.02 µg/ml) in Hanks' balanced solution was added. Two to four hours later, cells were harvested using a hypotonic solution of 0.075 M KCl and were incubated for 25 min at 37° C. A few drops of 1:3 mixture of glacial acetic acid and methanol fixative were added with gentle mixing. Cells were centrifuged for 10 min at 800 rpm and the cell pellet was resuspended in fixative. Three to four changes of fixative were made after centrifugation.

Standard procedure for slide preparation includes air drying at room temperature prior to trypsin treatment for G-banding and either air drying at room temperature or flame drying prior to Q-banding. Here we use one of three procedures: (1) air drying at room temperature, (2) flame drying (slides are dipped in 70% methanol and ignited), or (3) heating on a hot plate. Slides were then aged for two weeks to several months at room temperature.

Slides were treated with trypsin for 4 min at 37° C prior to staining. Trypsin stock solution consists of 1.0 gm Difco trypsin (1:250) dissolved in 400 ml of Dulbecco phosphate buffer solution (PBS) without Ca^{++} and Mg^{++}. Aliquots of 10.0 ml were frozen until needed. Working solutions contained 4 ml of the stock solution plus 40 ml of PBS without Ca^{++} and Mg^{++} warmed to 37° C.

G-banding procedure was that of Arrighi and Hsu (1974). Giemsa

stain solution consists of 2 ml of Gurr's improved R66 Giemsa stain with 2 ml of McIlvaines buffer at pH 6.8-7.0 and 36 ml of distilled water.

Results

The results are shown in Figures 1-3. Figure 1 shows four metaphase spreads from a flame dried slide which was aged several weeks before treatment with trypsin and Giemsa staining. Figure 1a shows G-banding with C bands evident in some chromosomes. Sister chromotids are swollen and united as is characteristic of G-banded chromosomes which have been trypsin treated. Chromosomes in Figure 1b are stained only at the periphery of the chromatids. This configuration has been explained as chromosomal collapse followed by an outward flow (Gormley and Ross, 1976). Chromosomes in Figure 1c show regular spiralling quite similar to that produced by hypotonic treatment. Sister chromatids are not swollen and are separated. Figure 1d shows strong C-banding. These different spiral and banding patterns are representative of those seen on many flame dried slides though the frequencies are variable.

The variety of chromosome morphologies seen on these slides is interpreted as reflecting differences in the temperature to which the cells were exposed. The temperature at which 70% methanol burns is approximately 375 °C, giving a drastic though brief heat shock. However, the flame and hence the heat are quite uneven over the surface of the slide. To control this variable heat, slides were placed for two minutes on a hot plate with controlled temperature. All other procedures were the same as for flame drying. The chromosomes shown in Figure 1 were reproduced in most cells of several slides at the following temperatures:

Figure	1a	1b	1c	1d
Temperature in °C	100	140-150	170-280	>300

Figure 1: Chromosomes which have been flame dried, aged for several weeks, trypsinized, and Giemsa stained.

a) G-bands

b) Collapsed chromosomes

c) Regular spirals

d) C-bands

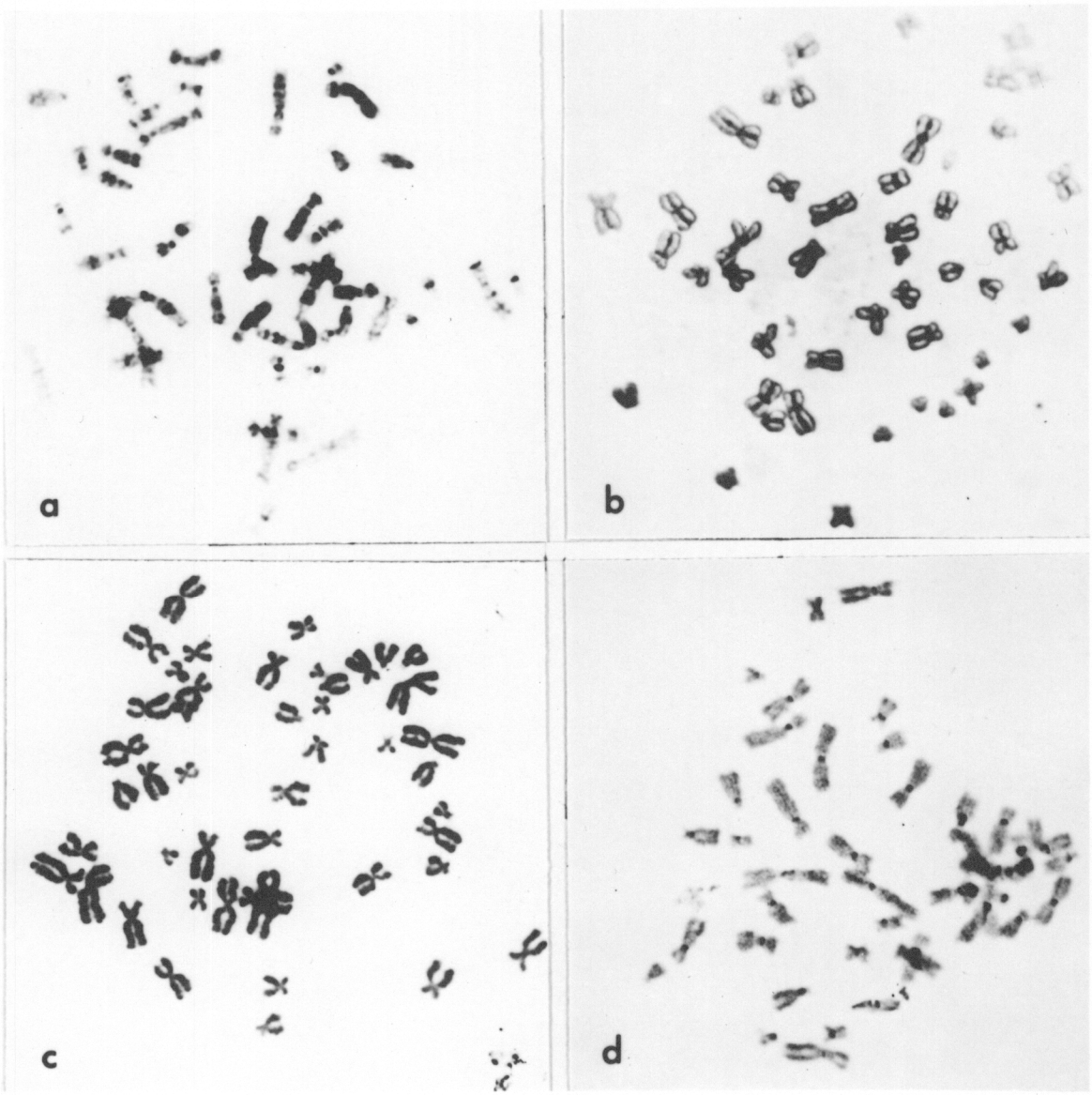


Figure 1

Figure 2: Chromosomes prepared as those in Fig. 1, but aged for several months.

a) Chromosomes with bands and spirals

b) Relaxed spirals

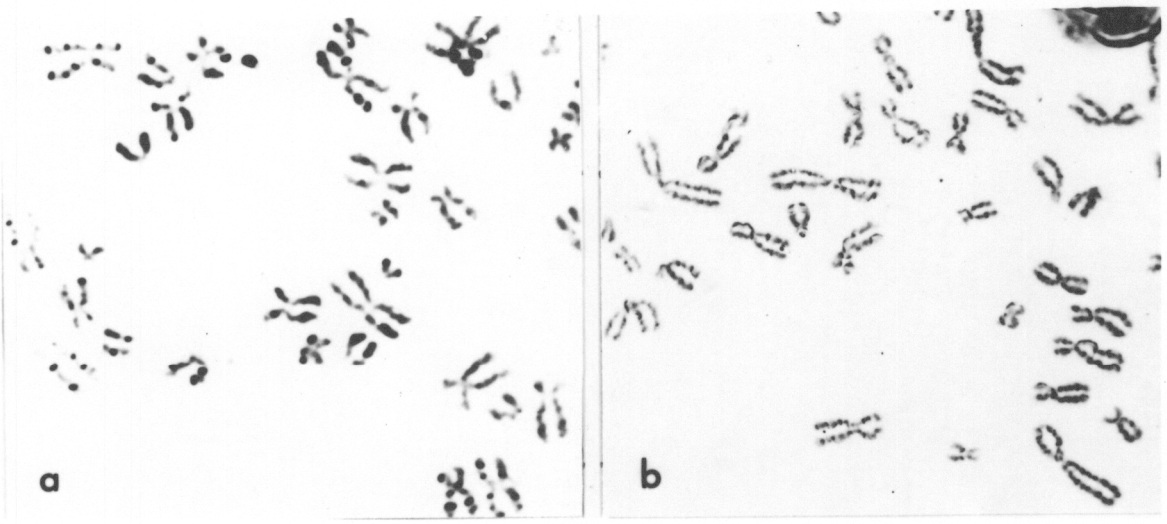


Figure 2

Figure 3: Conventional G-banded chromosomes prepared with air drying at room temperature rather than flame drying.

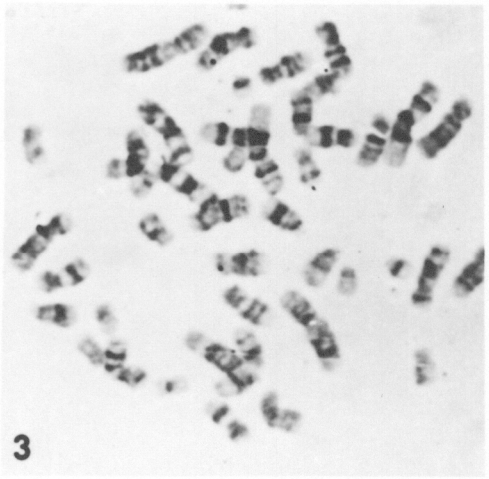


Figure 3

At temperatures above 300°C, the spread film did not remain intact on the slide. Thus, it was difficult to reproduce the C-banding shown in Figure 1d. We assume that it is produced at high temperatures since it does not occur at lower temperature ranges. These temperatures are approximate and can vary with factors such as the age of the slide.

Figure 2 shows metaphase spreads from a slide prepared in the same manner as the previous slide but aged for several months. Among metaphases similar to those in Figure 1 were two additional stages. Figure 2a is a transitional stage in which horizontal bands and spirals occur together. Sister chromatids often are asymmetrical. This finding is discussed more extensively in the next chapter. Figure 2b shows irregular relaxed spirals contrasted to the regular tight spirals of Figure 1c. These configurations have also been produced with quinacrine mustard staining.

Figure 3 shows conventional G-banding following air drying at room temperature and trypsin treatment. It is included here to show that spiralization resulted specifically from the combined treatment of heat and trypsin and not from other variables in our technique.

Discussion

The most common procedures for G-banding involve trypsin treatment of slides which have been air dried at room temperature. Q-banding procedures use no trypsin treatment and slides are either flame dried or air dried at room temperature. Neither heat nor trypsin alone is sufficient to produce spirals, but heat prior to trypsin treatment will produce spirals with either stain. Apparently, heat denaturation of chromatin makes available to the action of trypsin some chromosomal proteins which would otherwise be protected. The degradation of these proteins then allows the chromatin to assume a regular spiral configuration.

The difficulty in drawing inferences about native chromatin following pretreatment and fixation has been pointed out previously. In the extreme, Du Praw has argued that virtually all chromosome preparations are artifactual (1973). The usual fixation methods for banding remove some histones and non-histone proteins, making the DNA more accessible to external reagents (Dick and Johns, 1968; Sumner et al., 1973; Comings and Avelino, 1974). However, several lines of evidence suggest that banding does reflect normal chromosome structure: (1) Yunis and Sanchez have demonstrated banding without the usual pretreatments of heat, alkali or proteolytic enzymes (1973); (2) there is a strong correspondence of densitometric traces from electron micrographs of chromosomes with those from both Q- and G-banded chromosomes (Bahr et al., 1973; Ruzicka and Schwarzacher, 1974; Golomb and Bahr, 1974); (3) Ganner and Evans have shown a correspondence between late replicating regions of chromosomes and banding (1971). Thus, three quite different approaches support the

importance of banded regions as reflections of chromosome organization.

The present technique allows the study of the relationship between spirals and bands by varying the temperature. Of particular interest, is the intermediate stage with both spirals and bands (Fig. 2a). We believe that this technique will be useful in unravelling the nature of banding and the structure of chromosomes.

Summary

A new technique is described which produces spiralization of human metaphase chromosomes. The important feature is heat followed by trypsin treatment. By varying conditions, it is possible to produce bands, spirals and intermediate stages. This provides a new approach to the understanding of banding and chromosome structure.

Acknowledgments

We would like to thank Dr. Asim Esen for stimulating discussions Dr. Yogesh Goradia for constant encouragement.

References

Arrighi, F. E., Hsu, T. C.: Staining constitutive heterochromatin and Giemsa crossbands of mammalian chromosomes. In: Human chromosome methodology, J. J. Yunis, ed., pp. 59 - 71. New York: Academic Press 1974.

Bahr, G. F., Mikel, U., Engler, W. F.: Correlates of chromosomal banding at the level of ultrastructure. In: Chromosome identification technique and applications in biology and medicine, T. Caspersson, L. Zech, eds., pp. 280 - 289. New York: Academic Press 1973.

Comings, D. E., Avelino, E.: Mechanisms of chromosome banding. II. Evidence that histones are not involved. Exp. Cell Res. 86, 202 - 206 (1974).

Dick, C., Johns, E. W.: The effect of two acetic acid containing fixatives on the histone content of calf thymus deoxyribonucleoprotein and calf thymus tissue. Exp. Cell Res. 51, 626 - 632 (1968).

DuPraw, E. J.: Quantitative constraints in the arrangement of human DNA. Cold Spr. Harb. Symp. Quant. Biol. 38, 87 - 98 (1973).

Ganner, E., Evans, H. J.: The relationship between patterns of DNA replication and of quinacrine fluorescence in the human chromosome complement. Chromosoma (Berl.) 35, 326 - 341 (1971).

Golomb, H. M., Bahr, G. F.: Correlation of the fluorescent banding pattern and ultrastructure of a human chromosome. Exp. Cell Res. 84, 121 - 126 (1974).

Gormley, I. P., Ross, A.: Studies on the relationship of a collapsed chromosomal morphology to the production of Q- and G-bands. Exp. Cell Res. 98, 152 - 158 (1976).

Iino, A.: Observations on human somatic chromosomes treated with hyaluronidase. Cytogenetics 10, 286 - 294 (1971).

Kato, H., Yosida, T. H.: Banding patterns of Chinese hamster chromosomes revealed by new techniques. Chromosoma (Berl.) 36, 272 - 280 (1972).

Ohnuki, Y.: Demonstration of the spiral structure of human chromosomes. Nature 208, 916 - 917 (1965).

Ohnuki, Y.: Spiral structure of human somatic chromosomes. J. Cell Biol. 31, 82A (1966).

Ohnuki, Y.: Studies on the structure of somatic chromosomes, with special attention to spiralization. J. Cell Biol. 35, 154A - 155A (1967).

Ohnuki, Y.: Structure of chromosomes. I. Morphological studies of the spiral structure of human somatic chromosomes. Chromosoma (Berl.) 25, 402 - 428 (1968).

Osgood, E. E., Jenkins, D. P., Brooks, R., Lawson, R. K.: Electron micrographic studies of the expanded and uncoiled chromosomes from human leukocytes. Ann. N.Y. Acad. Sci. 113, 717 - 726 (1964).

Ruzicka, F.: Uber die ultrastruktur menschlicher metaphasenchromosomen. Humangenetik 17, 137 - 144 (1973).

Ruzicka, F., Schwarzacher, H. G.: The ultrastructure of human mitotic chromosomes and interphase nuclei treated by Giemsa banding techniques. Chromosoma (Berl.) 46, 443 - 454 (1974).

Shaw, M. W., Schwab, L. E., Brinkley, B. R.: Electron microscopy of human chromosomes. In: Perspectives in cytogenetics, the next decade, S. W. Wright, B. F. Crandall, L. Boyer, eds., pp. 251 - 282. Springfield (Ill.): Thomas 1972.

Sumner, A. T., Evans, H. J., Buckland, R. A.: Mechanisms involved in the banding of chromosomes with quinacrine and Giemsa. I. The effects of fixation in methanol-acetic acid. Exp. Cell Res. 81, 214 - 222 (1973).

Yunis, J. J., Sanchez, O.: G-banding and chromosome structure. Chromosoma (Berl.) 44, 15 - 23 (1973).

CHAPTER II

ASYMMETRY IN SISTER CHROMATIDS OF HUMAN CHROMOSOMES

Ranjan Y. Goradia

Brian K. Davis

The following paper has been selected for presentation at the Annual Human Genetics Meeting in LaJolla, California to be held October 21, 1977.

Introduction

Semiconservative replication produces two identical chromatids for each chromosome. The only reported cytological techniques which produce asymmetry between chromatids depend upon introducing that asymmetry during DNA replication [1,2]. These methods do not suggest any intrinsic asymmetry [3]. All of the many banding methods for metaphase chromosomes produce consistent patterns which are identical for sister chromatids and for homologs. The standard banding patterns are produced in human metaphase chromosomes when slides dried at room temperature are treated with trypsin and stained with Giemsa [4] or when slides (either flame dried or dried at room temperature) are stained with quinacrine mustard [5]. This report demonstrates that a combination of trypsin treatment and flame drying, followed by either quinacrine mustard or Giemsa staining, produces banding differences between sister chromatids and often between homologs.

Materials and Methods

Chromosome preparations were obtained from leucocyte cultures. Dulbecco's modified Eagle's medium was supplemented with 15% calf serum, penicillin-streptomycin, and phytohemagglutinin. Growth was arrested after 72 hours by adding colchicine (0.02 µg/ml). A hypotonic solution of sodium citrate was used. Chromosomes were washed and fixed in 3:1 methanol-acetic acid mixture. Drops of fixed material were dropped on slides dipped in 70% methanol and the slides were ignited. They were then aged for several months.

The aged slides were immersed in a Coplin jar containing a solution of 0.025% trypsin in PBS at 37°C for four minutes. The trypsin action was stopped by dipping the slides in absolute ethanol. Slides were then stained with quinacrine mustard and visualized using a fluorescent microscope or stained with Giemsa and observed by phase microscopy. Kodak Tri-X film was used for photomicrography.

Results

The surprising finding was that chromosomes showed asymmetry of bands in sister chromatids. Figs. 1A and B show overlapping sections from the same metaphase stained with quinacrine mustard. The majority of chromosomes have sister chromatids asymmetrical in the number of bright bands and their positions. This is less clear in smaller chromosomes. Some Q-bands are oblique and almost all of the bands appear to have a larger diameter than the rest of the chromatid.

Fig. 1C shows two enlarged number 6 chromosomes from Figs. 1A and B. Although the sister chromatids have dissimilar banding patterns, the homologs show similar patterns. Thus, each chromatid is more like its homologous chromatid than it is like its sister chromatid. In contrast, Fig. 1D shows chromosome 2 from Fig. 1A in which there is a breakdown of banding symmetry in the homologs as well as in the sister chromatids. None of the four chromatids have the same banding pattern.

A karyotype in Fig. 2 contrasts the usual Q-banding following flame drying and quinacrine mustard staining [5], with banding produced by our technique. For each of the 23 chromosome types, one control chromosome appears on the left with the two homologs from an experimental metaphase on the right. The experimental chromosomes show asymmetry in sister chromatids and between homologs as seen in Fig. 1. In most cases the distorted bands show some similarity to control bands. This point is developed below.

In order to compare this unusual banding pattern between cells and with normal banding, we show chromosome 1 from six experimental cells

Figure 1: Human chromosomes prepared by flame-trypsin treatment and stained with quinacrine mustard.

A. and B. - A metaphase spread. Arrows indicate chromosomes shown in C and D.

C. Enlarged number 6 chromosomes from the metaphase of A and B.

D. Enlarged number 2 chromosomes from the metaphase of A and B.

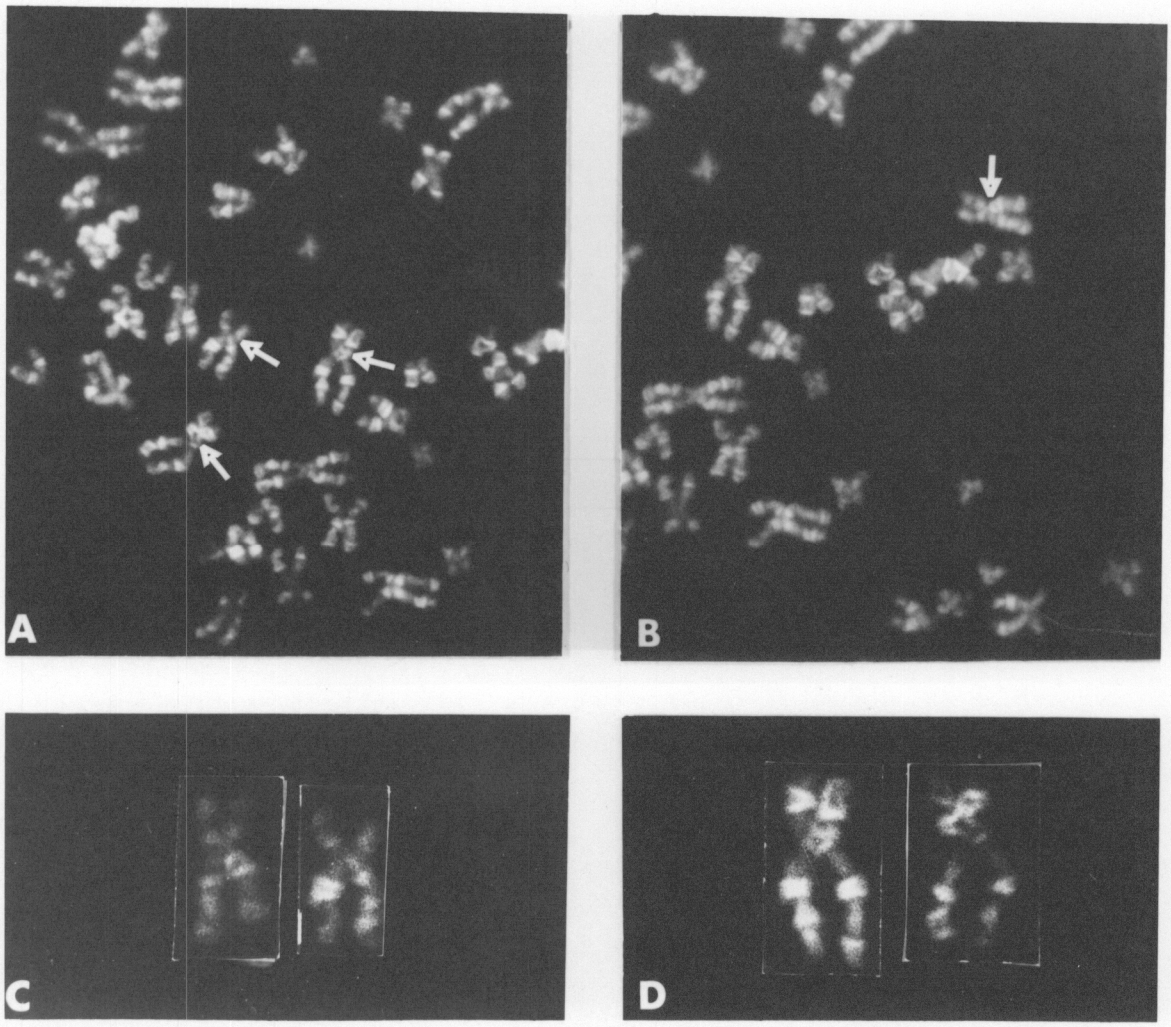


Figure 1

Figure 2: A karyotype of human chromosomes stained with quinacrine mustard. For each chromosome type, one control chromosome is on the left with the two homologs from a cell prepared by the flame-trypsin method.

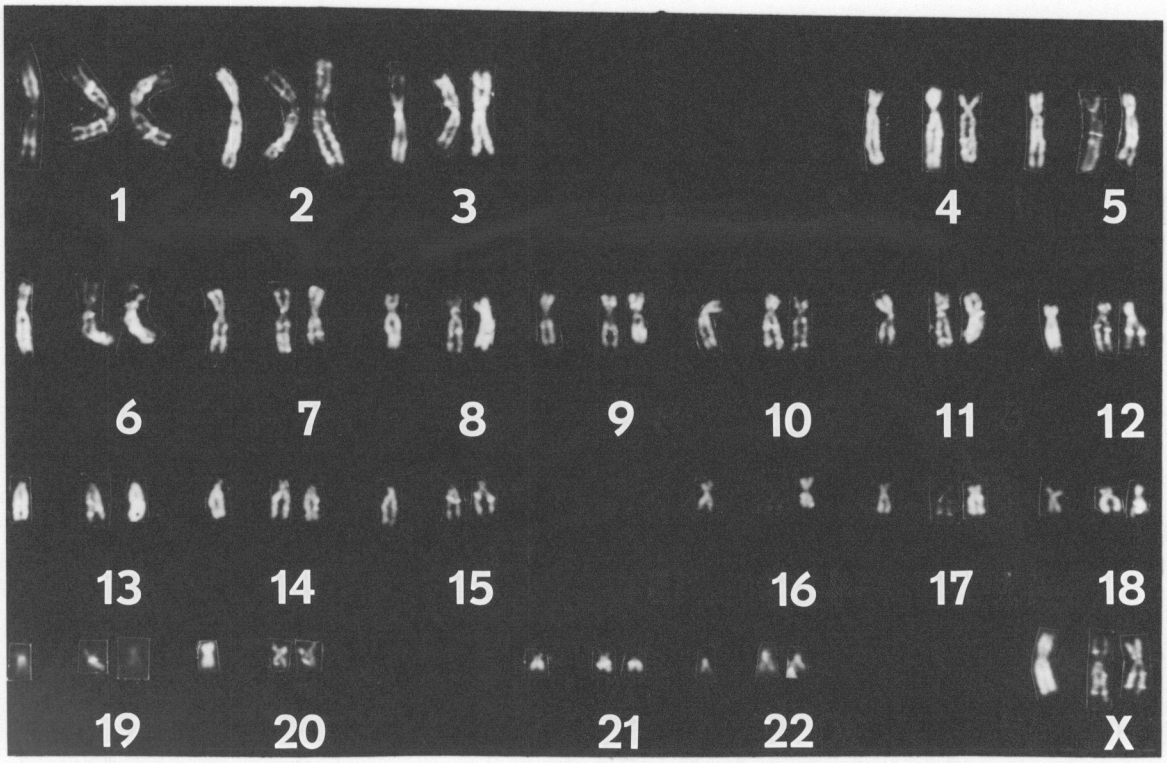


Figure 2

stained with quinacrine mustard (Fig. 3B-G) and one experimental cell stained with Giemsa (Fig. 3H). The two chromosomes from a control cell appear in the upper left (Fig. 3A). The typical banding pattern of two bands in the short arm and five bands in the long arm [6] can be seen in these control chromosomes. Experimental chromosomes show fewer bands and these are often displaced and oblique. Although the chromosomes vary, some patterns are repeated. The most common feature of the short arm is a distinct band in one chromatid with an offset band in the sister chromatid. This is clearest in the left chromosomes of 3D, F and G. The long arms are much more variable, though some have symmetrical sister chromatids (e.g. both chromosomes of 3B). All of the 14 experimental chromosomes are asymmetrical in at least one arm.

Figure 3: Number 1 chromosomes from eight different cells.

A. Chromosomes showing standard quinacrine banding, without trypsin treatment.

B-G. Chromosomes prepared by flame-trypsin treatment and stained with quinacrine mustard.

H. Chromosomes prepared by flame-trypsin treatment and stained with Giemsa.

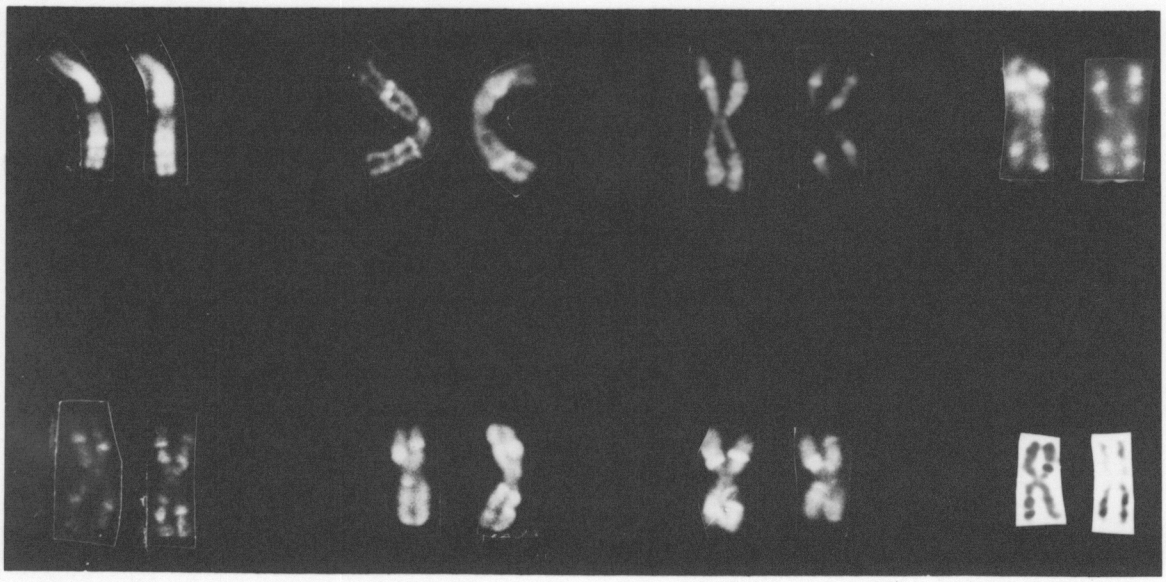


Figure 3

Discussion

Apparently, the combined effect of flame drying and trypsin produces chromatin patterns which are intermediate between banding and spiralization and asymmetrical in sister chromatids. The relationship between banding and spiralization is discussed elsewhere [7]. It is unlikely that the asymmetry demonstrated here results from differences in chromatin composition of sister chromatids. There are at least two ways of generating asymmetry without compositional differences. The first possibility is that each chromatid is asymmetrical around its circumference. Sister chromatids would appear asymmetrical if they presented different surfaces on the slides. A second possibility is that each chromatid has optional structural configurations. The choice of options might be directed, for example, from the centromere, such that sister chromatids assume different configurations. It has been shown [8,9] that although spiralization produced by hypotonic treatment has the same pattern in sister chromatids, it is often in opposite directions. That is, chromosomes have bilateral symmetry. These differences between sister chromatids could be important in regular disjunction at anaphase by ensuring recognition by spindle fibers so that fibers from the same pole do not attach to both sister chromatids.

Summary

Human metaphase chromosomes show identical lateral bands in sister chromatids when stained with quinacrine mustard or Giemsa-trypsin. A hybrid of these two methods produces banding patterns which are different in sister chromatids yet may be repeated in homologous chromatids.

Acknowledgments

We would like to thank Dr. Asim Esen for stimulating discussions, Dr. G. F. Bahr for helpful comments, and Dr. Yogesh Goradia for constant encouragement.

References

1. Latt, S. A., Proc. Nat. Acad. Sci. U.S. 70, 3395 (1973).

2. Taylor, J. H., Woods, P. S., and Hughes, W. L.; Proc. Nat. Acad. Sci., U.S. 43, 122 (1957).

3. Dutrillaux, B., Aurias, A. and Fosse, A. M.; Exp. Cell Res. 97, 313 (1976).

4. Dutrillaux, B.; Chromosome identification - techniques and applications in biology and medicine (T. Caspersson and L. Zech, eds.) p. 38, Academic Press, New York (1973).

5. Caspersson, T., Zech, L. and Johansson, C.; Exp. Cell Res. 62, 490 (1970).

6. Paris Conference (1971): Standardization in human cytogenetics. Birth Defects: Original Article Series, VIII: 7. The National Foundation, New York (1972).

7. Goradia, R. Y. and Davis, B. K.; Hum. Genet. 36, 155-160 (1977).

8. Osgood, E. E., Jenkins, D. P., Brooks, R. and Lawson, R. K.; Ann. N.Y. Acad. Sci. 113, 717 (1964).

9. Ohnuki, Y.; Chromosoma 25, 402 (1968).

CHAPTER III

SEX CHROMOSOME MOSAICISM IN A MOTHER AND TRISOMY 13 IN HER CHILD

Ranjan Y. Goradia

Brian K. Davis

John Mann

The following paper has been accepted for publication in the Proceedings of the Birth Defects Conference held in Memphis, Tennessee in June, 1977.

I. Human Trisomies

A. Trisomy X

Although the first reported case of trisomy X or triplo-X (47,XXX) was quite abnormal in phenotype[1,2] it has subsequently become clear that this is atypical. The majority of individuals are physically normal, which would be expected from the mechanism of X-inactivation. There is extreme variability, ranging from normality to extreme cases such as the original one. The same variability and overlap with normality are found for mental ability. The risk of mental abnormality remains uncertain.[3,4] Because of the phenotypic variability it has been impossible to define a trisomy X syndrome in the clinical sense.[33]

The extra X chromosome may be present in all cells (complete trisomy X) or only in some fraction of cells (mosiac or mixoploid). In the latter, there are several variables: 1) the frequency of aneuploid cells, 2) the number of cell types, and 3) the distribution of cell types in the body. The frequency of aneuploid cells varies over a large range.[3] The question of what fraction of aneuploid cells must be found to classify an individual as a mosaic rather than a normal with a few aneuploid cells, ramains unresolved. The simplest mosaics have two cell types, 46,XX and 47,XXX, but monosomic, tetrasomic and/or pentasomic cells may also be present.[3] The distribution of cell types has been shown, by karyotypes and by Barr bodies, to vary from tissue to tissue and even from left to right within the same tissue.[5] The phenotype of mosaics would thus be expected to be extremely variable and indeed it ranges from apparently normal to quite abnormal. Since the phenotypic distri-

butions are indistinguishable, most authors consider complete trisomy X and mosaic trisomy X together.

Examination of interphase nuclei for the presence of two Barr bodies is a useful method for identifying individuals with at least three X chromosomes. It must be remembered that many nuclei of 46,XX females do not show a Barr body, and similarly, most nuclei of trisomy X females do not show both Barr bodies. Buccal smear analysis of the first reported case showed 57 percent of cells with one sex chromatin body and 14 percent with two.[1] The average frequencies for buccal smears from 125 triplo-X individuals, as compiled by Barr et al.,[3] were 43% with one body and 24% with two. The sample included 108 complete trisomies and 17 mosaics. As expected, the mosaics had variable frequencies of Barr bodies, scattered all through the range of the entire group, but on the average, fewer cells showed Barr bodies (38% with one and 16% with two). The failure of early surveys to detect any double chromatin-positive newborn girls, cited by Hamerton,[6] may not be surprising in view of these frequencies.

Some properties of trisomy X compared to other trisomies are given in Table 1.

B. Trisomy 13

Trisomy 13 (47,XX,+13 or 47,XY,+13), also called trisomy D_1, Patau's Syndrome or occasionally Bartolin-Patau Syndrome, would be expected to be more deleterious than any of the other three common trisomies since trisomy 18 and trisomy 21 involve smaller chromosomes and trisomy X is modified through X inactivation. The severity of the trisomy 13 syn-

Table 1

Severity Index of Human Trisomies

Trisomy:	13	18	21	X
Phenotype	Grossly Abnormal	Grossly Abnormal	Abnormal	Variable
Fifty percent post-natal mortality	one month[a]	one month[b]	3-4 years[c]	Unknown[d]
Percent among live births[e]	0.005	0.012	0.125	0.104[f]
Percent of total trisomies among spontaneous abortions[g]	0.033	0.057	0.090	0.005[h]
Percent of total trisomies among live births[i]	0.017	0.045	0.416	0.347[h]

a. Magenis and Hecht[7]

b. Hecht[8]

c. Miller[9]

d. Probably not reduced greatly from XX females

e. Hook and Hamerton[10]

f. Frequency of trisomy among live born females only

g. Calculated from data in Carr and Gedeon[11]

h. Includes both XXX and XXY

i. Jacobs[12]

drome is indicated by the following: 1) it is rare among live births, 2) the phenotype involves gross mental and physical defects,[7] 3) the life expectancy is extremely short,[7] and 4) among spontaneous abortions, trisomy 13 accounts for 1.70 percent of total chromosome anomalies (calculated from a recent review by Carr and Gedeon[11]). There are nine other trisomies, including both 18 and 21, which are more common in the samples. However, Jacobs[12] has compared the relative frequencies of each chromosomal trisomy among abortions with trisomies and liveborn with trisomies. Her results show that chromosome 13 is more prevalent among aborted trisomies than among liveborn trisomies, whereas the converse is true for both chromosomes 18 and 21. We have made the same comparisons using abortion data which are more recent and extensive,[11] and find that while the percentages have changed somewhat, particularly for chromosome 18, it remains clear that trisomy 13 is much more likely to be aborted. These comparisons for trisomies 13, 18, 21, and X are summarized in Table 1.

Taylor et al.[13] have presented three new cases and reviewed the chromosomal etiology of trisomy 13 from the literature. They found 87 (71%) complete trisomies, 25 (20%) unbalanced translocations, and 11 (9%) mosaics among the total 123 cases. This is in reasonably close agreement with the earlier surveys of 27 cases by Taylor[14] and 221 cases by Magenis, et al.[15] Of the eleven mosaics reported by Taylor, et al.,[13] only five were of the simplest type, 47,XX/47,XX + D or 46,XY/47,XY + D. The other six cases were mosaic for chromosome rearrangements or in one instance, tetrasomy as well as trisomy, and included three infants with-

out a normal cell line.

Here we report on what we believe is a unique case, in which a mother with mosaic trisomy X has produced a child with complete trisomy 13.

II. Investigation of a Family with Two Trisomies

A. Case report

The proposita, a 31 year old white woman, was found to be in good health and physically normal except for some evidence of ovarian dysfunction. Menarche was at age 13, with very irregular menses. She married at age 25 and took birth control pills for the following year. At age 29 she began fertility evaluation after failure to become pregnant. Her ovulation was unpredictable. At age 30 she took the fertility drug Clomid for six months.

She became pregnant and delivered a 5 lb. 14 1/2 oz. male infant approximately two weeks prematurely. Multiple anomalies noted at birth included short depressed palpebral slants, microphthalmia, micrognathia, wide nasal bridge, small low set and poorly molded ears, increased nuchal folds, a soft palate cleft, an unbilical hernia, a single unbilical artery, elongated thumb and some overlapping of fingers, bilateral simian crease, ulnar polydactyly, rocker-bottom feet, anal stenosis, undecended testes, and hyperconvex nails. The face of the child is shown in fig. 1. The eyes were small, each less than one cm in diameter, and placed deeply in the orbits. The left side view of the head (fig. 2) shows the low set malformed ears. The right hand (fig. 3) shows a simian crease and a small sixth digit. The rocker-bottom right foot is shown in fig. 4. The diagnosis was trisomy 13. The infant expired twelve days after its birth. The autopsy examination confirmed the features listed above and also found the left kidney to be absent.

Figure 1: The face of the trisomy 13 infant.

Figure 2: The left side view of the head of the trisomy 13 infant.

Figure 3: The right hand of the trisomy 13 infant.

Figure 4: The right foot of the trisomy 13 infant.

Figure 1

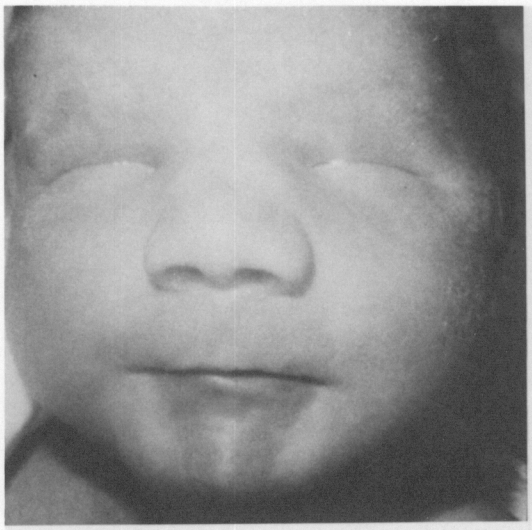
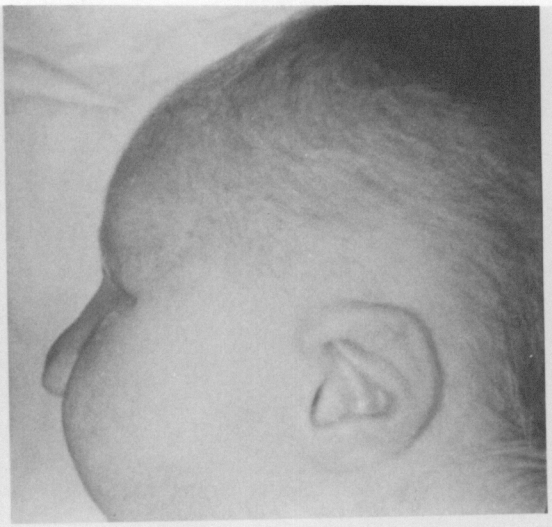


Figure 2



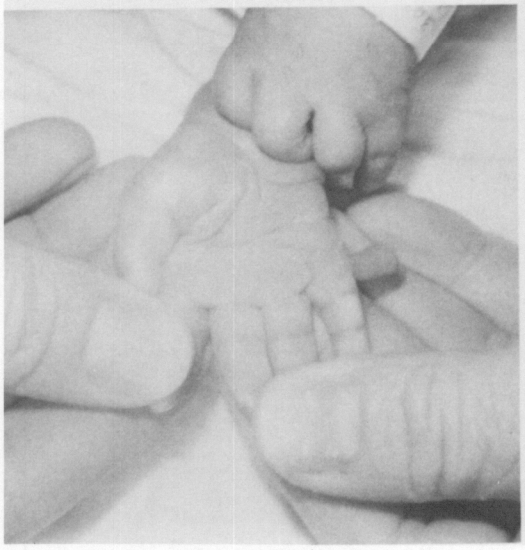


Figure 3

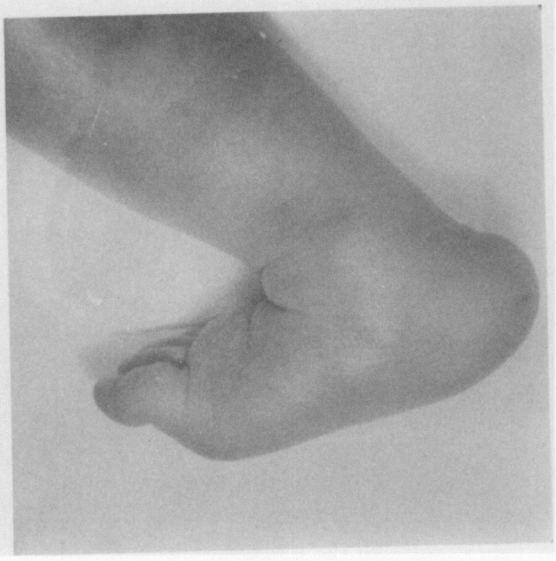


Figure 4

The pedigree of the proposita's family is shown in fig. 5. There is no known consanguinity. She was the youngest of nine children. Three brothers (II-3, II-5, II-12) have produced eight normal children. All five sisters have had normal menses. Two unmarried sisters (II-9, II-14) and one married sister (II-16) have no children. Another sister (II-8) has had two normal daughters and two miscarriages. The remaining sister (II-11) has three normal daughters and a son (III-12) with optic agenesis and cutis marmorata. Among the surviving children of the nine siblings of the second generation are ten females and four males. The mother of the proposita (I-3) died at age 42 of childbirth complications and the father (I-2) died at age 68 of silicosis. The family history of the husband of proposita (II-17) was unexceptional.

B. Chromosome Analysis

1. Materials and Methods

Leucocytes were cultured from the proposita, her husband and their child, using Dulbecco's medium supplemented with fetal calf serum, antibiotics, and phytohemagglutinin. Cells were treated with colchicine and harvested after 72 hours, using 0.075 M KCl as hypotonic solution and a 3:1 methanol-acetic acid fixative. Chromosomes were banded with Giemsa or quinacrine for karyotype analysis.

Buccal smears from the proposita were stained with orcein to look for Barr bodies or with quinacrine to look for Y bodies.

2. Results

Twenty metaphase spreads from the husband of the proposita were counted and were all the normal 46,XY (fig. 6). Twenty metaphase

Figure 5: The pedigree of the family. The mosaic trisomy X proposita is indicated with an arrow.

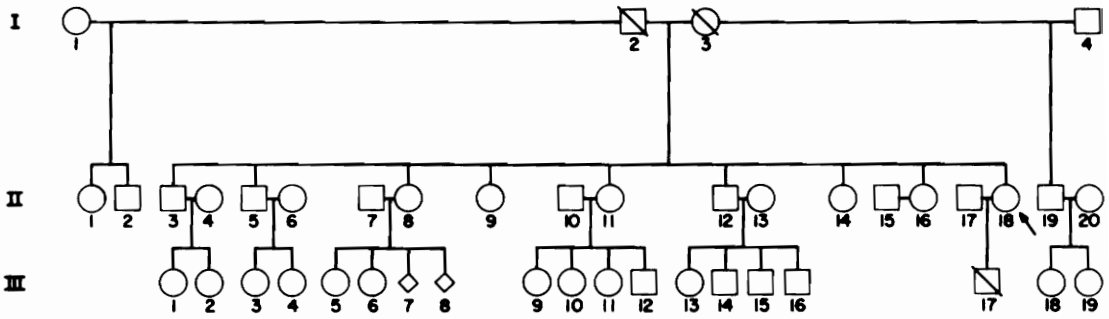

I
1
2
3
4
II
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
III
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Figure 5

spreads from the child uniformly showed 47 chromosomes. Banding identified the extra chromosome as number 13 (fig. 7), thus confirming the diagnosis of trisomy 13 based on clinical features.

After an initial indication of mosaicism in the proposita's cells, 200 metaphase spreads were counted. One hundred and eighty-two (91%) of the cells were normal 46,XX, but the remaining eighteen (9%) had one or two extra chromosomes. Figure 8 shows one of these metaphase spreads with two normal X chromosomes and a chromosome which is about ten percent longer than the normal X chromosomes, is partially uncoiled near the centromeric region of the long arm and twisted near the centromeric region of the short arm, and has the banding pattern of an X chromosome. This distinctive morphology along with clear X banding was found in five of the eighteen aneuploid cells. The other thirteen cells had a variety of smaller chromosomes, including rings and fragments which we believe were derived from the extra X chromosome, though in most cases banding is inconclusive. In only one cell was the extra chromosome the size of a D chromosome and banding did not suggest chromosome 13. The mosaicism involves an unstable X chromosome and not chromosome 13 for which her child is aneuploid. Complete cytogenetic analysis of this X chromosome is detailed in Appendix A.

Buccal smears from the proposita showed 39 percent of the cells with one Barr body and none with two Barr bodies. This is not surprising since karyotype analysis showed that only 2.5 percent of cells (5/200) have a large extra X chromosome, and since as it was noted earlier, the fraction of cells showing two Barr bodies in trisomy X

Figure 6: A metaphase spread from a leucocyte of the proposita's husband with the normal 46,XY constitution.

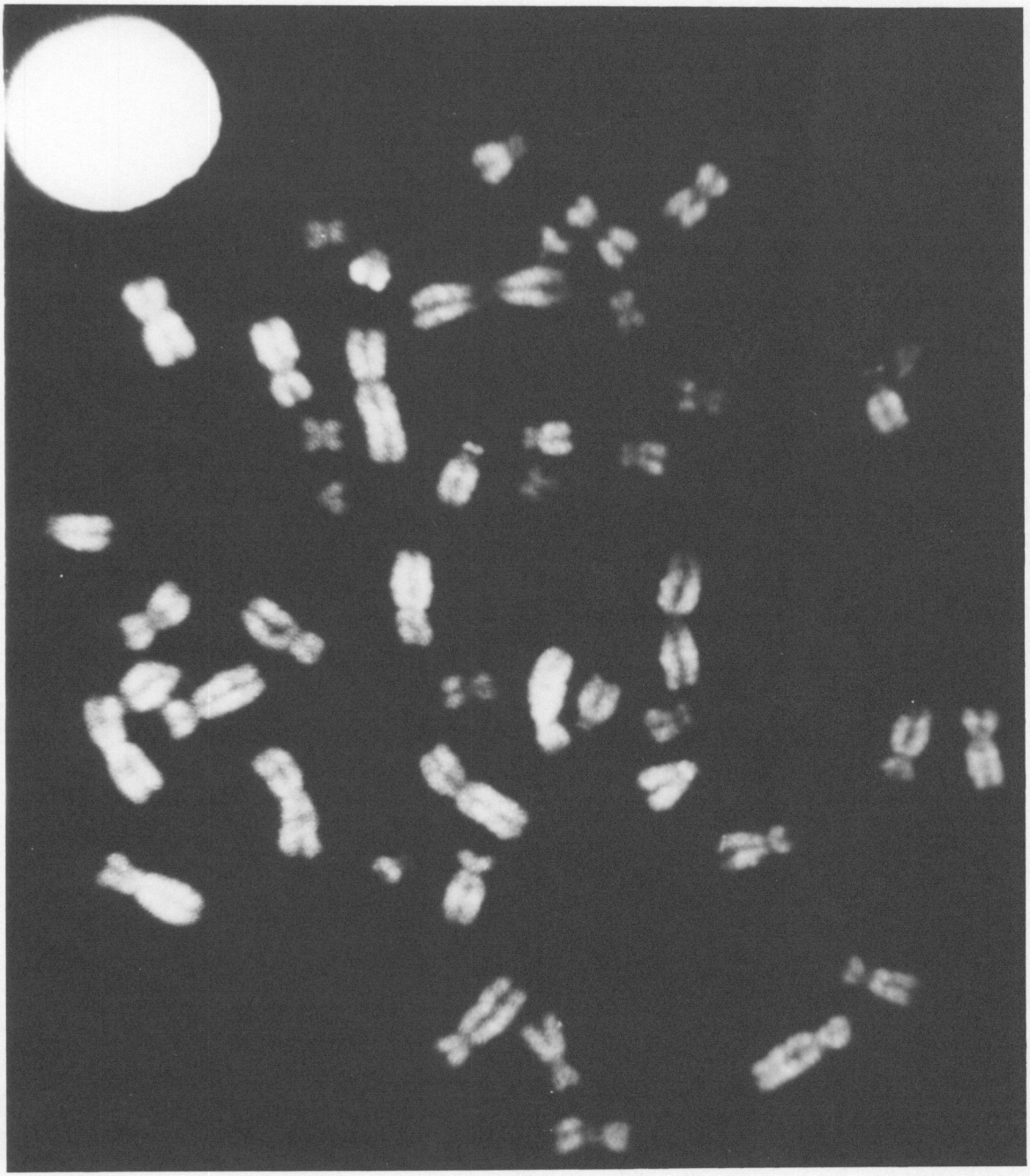


Figure 6

Figure 7: A metaphase spread from a leucocyte of the trisomy 13 infant. Arrows point to the three number 13 chromosomes.

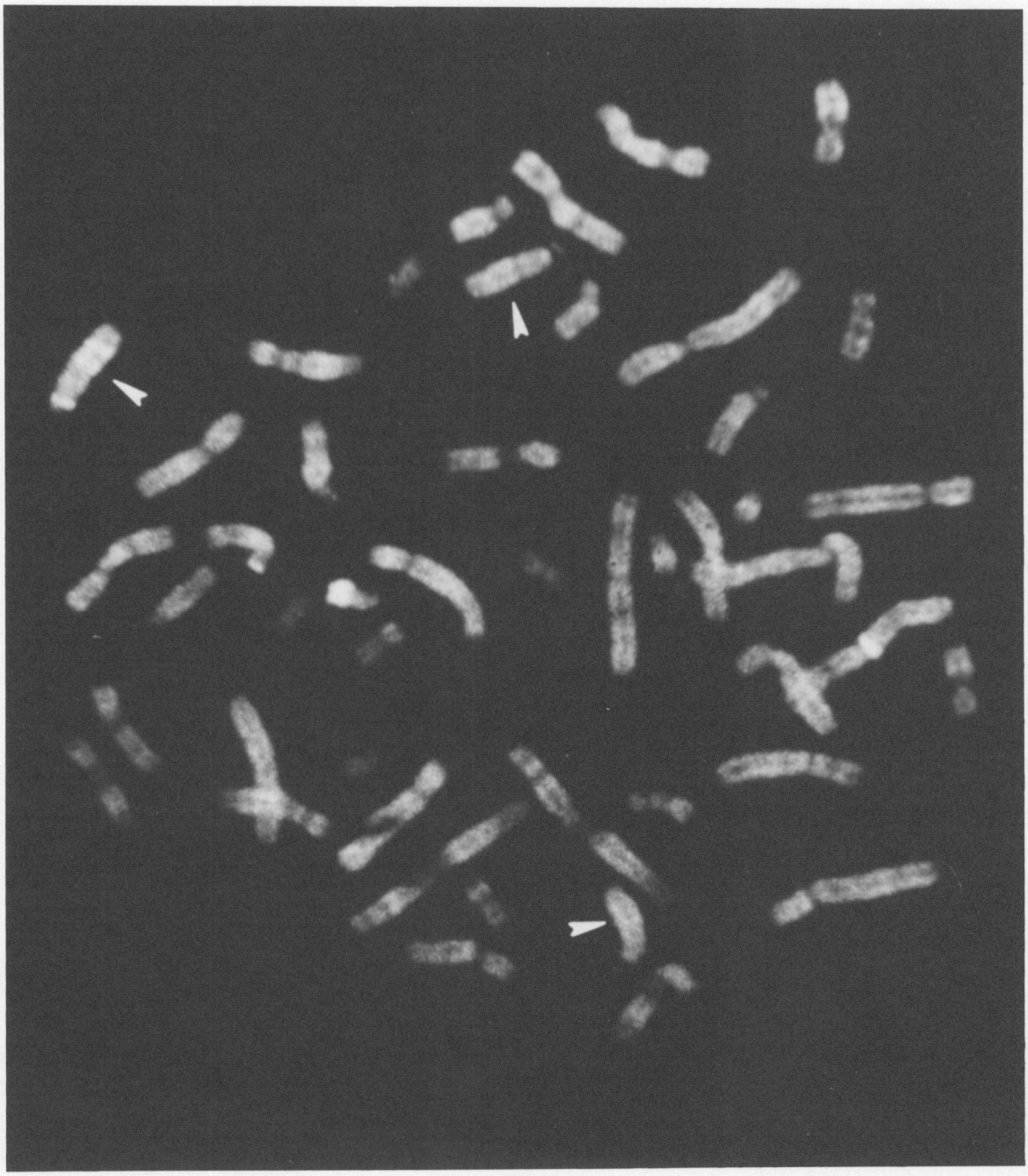


Figure 7

Figure 8: A metaphase spread from an aneuploid leucocyte of the proposita. Arrows point to the three X chromosomes.

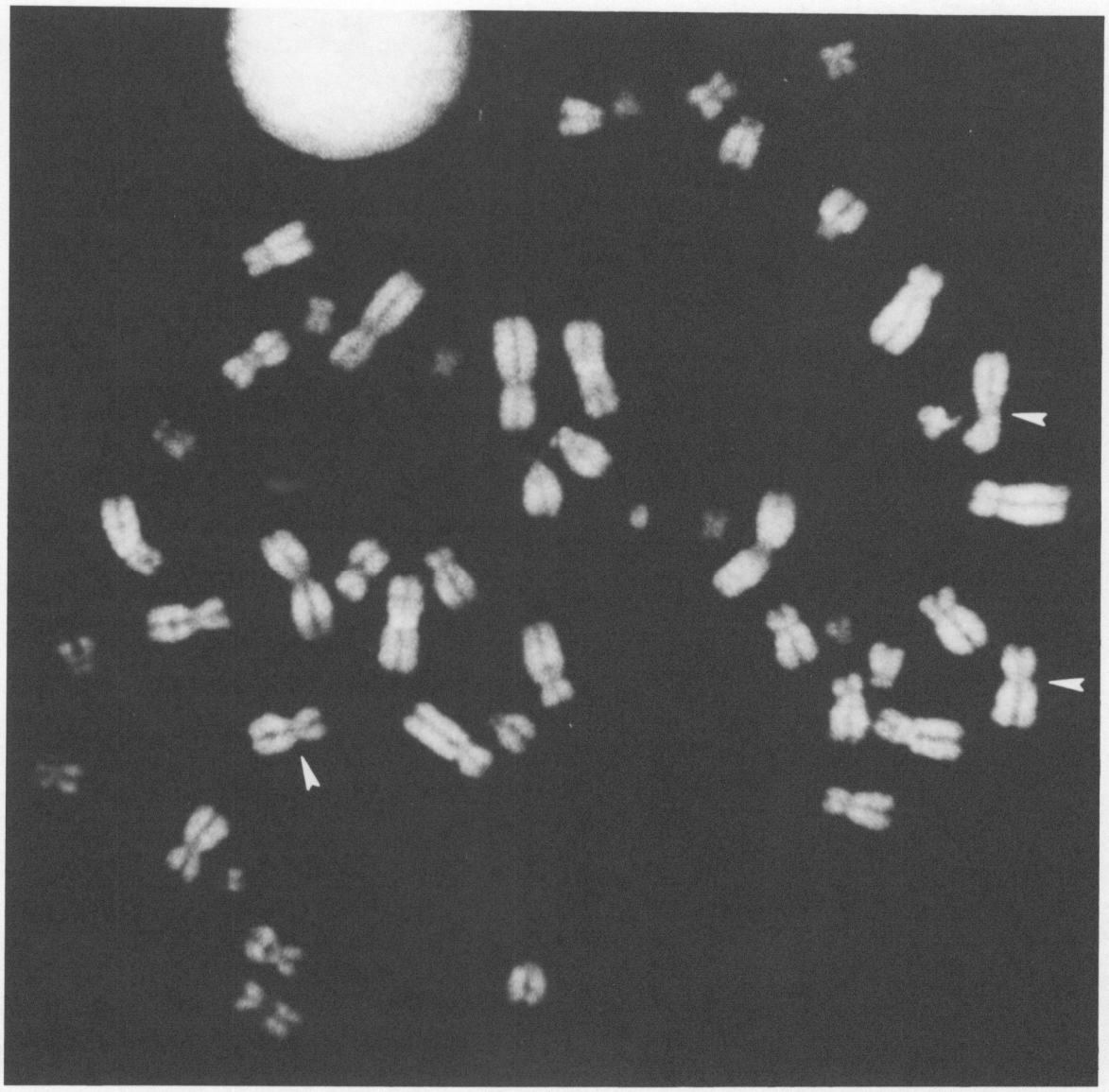


Figure 8

Figure 9: An inked print of a pocket whorl from the proposita.

Figure 10: An inked print of the double loop whorl of the proposita.

Figure 9

Figure 10

mosaics averages only 16 percent and is quite variable. Staining for Y bodies was negative in all cells.

C. Dermatoglyphics

The total finger ridge count of the proposita was 113, which is closer to the average count of 109.8 for trisomy X females of British extraction than to the average count of 127.2 for normal females,[16] although the ranges of both groups are so broad that 113 is common for both.[16,17] The lower ridge count of the proposita did not result from the presence of arches. She had five loops and five whorls, of which three were pocket whorls (fig. 9), one was a spiral whorl, and one was a double loop (fig. 10). In normal females, the mean ridge count for loops is 12.1 and for whorls is 18.3.[17] The proposita had corresponding counts of 11.8 and 11.0, showing that her lower total ridge count resulted primarily from the pocket whorls.

III. Inheritance of Trisomies

A. Progeny of Trisomies

If disjunction of the three homologs in a trisomic is random, then one expects one half normal monosomic gametes and one half disomic gametes. This will result either from trivalent pairing or from formation of a bivalent and univalent, provided the univalent is not lost at anaphase. If fertilization is also random and survival in unaffected, then one half normal disomic progeny and one half trisomic progeny are expected. In Drosophila melanogaster, triplo-X females rarely survive and are sterile when they do,[18] but triplo-4 flies usually, though not always,[19,20] produce the expected 1 disomic:1 trisomic progeny ratio. The behavior of trisomics in plants has been thoroughly reviewed by Khush.[21] In most species, the extra chromosome is transmitted in less than the expected 50% frequency. Khush has detailed the evidence for the mechanisms which have been proposed to account for this.

In humans, trisomy 21 males are sterile, but there are a number of reports of fertile trisomy 21 females. Their offspring show a slight deficiency of affected individuals, though the numbers are small.[4,6,22,23,24] As Mikkelsen has pointed out,[23] some deficiency is expected since trisomy 21 fetuses suffer a high spontaneous abortion rate. Since trisomies 13 and 18 do not survive to reproductive age, the behavior of the three homologs in gametogenesis must be examined in a different way. Mosaics with a low frequency of trisomic cells might be phenotypically normal, and yet have a high risk of producing children with complete trisomies. Finding such mosaics among the parents of

trisomic children would support this idea. The fact that they have not yet been found for trisomies 13 or 18[13,14] does not rule out this possibility. Mosaicism for trisomy 21 has been detected in both fathers and mothers after the birth of a Down's syndrome child.[25]

The situation with trisomy X in humans is somewhat more complex. As usual, randomness predicts 1 monosomic:1 disomic gamete and 1 disomic:1 trisomic zygote, but fertilization by X- or Y-bearing sperm should produce four types of progeny with equal frequency: 46,XX; 46,XY; 47,XXX; and 47,XXY. The pooled results from numerous reports show all four of these classes, but with only a few of either trisomy and with an excess of normal males over normal females.[3,4,6,22] The largest compilation, that of Barr et al.,[3] summarized the information on 67 children of 28 trisomy X women. Of the children whose sex was recorded, 31 were male and 19 were female. Twenty-nine children who were tested cytogenetically for sex chromosome constitution consisted of three males with 47,XXY, one male with 46,XY/47,XXY mosaicism, one female with 46,XX/47,XXX mosaicism, and 24 with normal sex chromosome constitutions. The authors have pointed out the interesting feature that four of the five women who did produce aneuploid offspring were themselves mosaic, even though only eight of the total 28 women in the sample were mosaic.

The preponderance of normal progeny from trisomy X mothers is usually attributed to meiotic drive. However, the excess of normal sons over normal daughters can only be accounted for by a post-meiotic event, either sperm selection or differential fetal survival. The

uterine environment of normal 46,XX females is compatible with development of 47,XXX and 47,XXY fetuses, as evidenced by the scarcity of these karyotypes among spontaneous abortions.[11] We are led to postulate that the uterine environment of trisomy X females is such that the successful survival of the fetus depends upon its sex chromosome complement and that a mosaic female is likely to have a more normal uterine phenotype. While aneuploid zygotes will be produced by both mosaic and complete trisomy X females, they are much more likely to complete development with a mosaic mother.

B. Families With More Than One Trisomy

The previous section discussed the inheritance of a trisomy from a parent who has the same trisomy. Here the discussion is broadened to include inheritance of a trisomy from a parent with a different trisomy and the occurrence of trisomies in other relatives. These numerous cases have been reviewed[6,23,26,27] and presently are interpreted as showing a clearly nonrandom clustering of aneuploids.

Part of the nonrandomness results from the correlation of trisomy with maternal age. A second factor is the balanced translocation which generates unbalanced aneuploids with regularity. A third cause is mosaicism in parents. In some families the mosaicism has been difficult to demonstrate,[25,28] suggesting that undiscovered mosaicism might account for many cases of familial chromosomal aneuploidy. However, if a correction is made for maternal age, if translocation aneuploids are eliminated, and if one considers only families in which individuals have different chromosomal trisomies such that direct transmission from

Table 2

Combinations of two trisomies which have been reported either as a double trisomy or within close relatives.

	13	18	21	X
13	+[a]	+	+	-[b]
18	-	+	+	
21	+	+		
X	+			

a. A "+" indicates that the combination has been reported. Mosaics were included.

b. A "-" indicates that the combination has not been reported.

a mosaic parent cannot be an explanation, then there remains a nonrandom risk of producing a trisomic conceptus.[12] It is therefore important to accumulate information on cases such as the one in this report.

C. Double Trisomy

A double trisomy is a type of multiple aneuploidy in which an individual rather than a family has more than one trisomy. There are many examples in the literature (for reviews, see 4, 6, and 23). It has been argued that the frequency of double trisomies for XXY and trisomy 21 indicates nonrandomness[29] just as is found for multiple trisomies within families. More recent data have shown that this is true for newborns but not older groups, indicating high mortality of the double trisomy.[23,30]

We have found examples in the literature of individuals or of families or of both, having eight of the ten possible pairs of trisomies for chromosomes 13, 18, 21 and X (Table 2). We have included mosaics of all types, including the interesting report by Baikie et al.[31] of a 47,XX,+D/47,XX,+E/48,XX,+D+E female. The two pairs of trisomies which we did not find are trisomy 18 with trisomy 18 and trisomy X with trisomy 13. Here we report on a family with the latter pair, and we are preparing a report on an example of the former pair.

D. Further Evidence for Nonrandom Trisomies in Families

Jacobs has presented two further arguments for an increased probability of trisomies in certain families.[12] The first line of evidence is the demonstration of a highly significant concordance between the chromosome status of two consecutive karyotyped abortions in 70 women.

If one abortion has a normal karyotype, the second is more likely to also. Conversely, if one shows a chromosomal abnormality, the second is more likely to be chromosomally abnormal, though not necessarily having the same abnormality. This seems to involve principally trisomies, and trisomies C and D in particular.

The second argument comes from the demonstration by Alberman et al.[32] of a high probability that women who spontaneously aborted a trisomy previously had a trisomy 21 child. This is not true for women who aborted a chromosomally normal fetus or even for those who aborted a fetus with a chromosomal abnormality other than a trisomy.

IV. Interpretation of the Family Presented in the Report

There are three plausible explanations which could account for a woman who is mosaic for trisomy X producing a trisomy 13 child. The first is coincidence. The second is the interference of pairing between homologs by a nonhomologous chromosome. The third is the presence of a cell division mutant.

Although we have presented at some length the evidence for nonrandom occurrence of trisomies, it is impossible to rule out coincidence in any particular family with two trisomies. Having ascertained that the child of proposita has trisomy 13, the proposita is expected to have trisomy X with the same probability as any other female, about 0.00104.[10] This cannot be tested with one example. However, the four-year failure of this woman to become pregnant, after stopping the use of birth control pills, raises the possibility of earlier conceptions with chromosomal anomalies which may have been aborted before they became recognizable pregnancies. Therefore, we consider two explanations which assume a direct relationship between the two trisomies.

The presence of an extra X chromosome in primary oocytes may interfere with the pairing of other chromosomes, in this case number 13, to cause nondisjunction. Mikkelsen has suggested such a mechanism to account for familial associations which occur frequently, such as trisomy 21 with DqDq translocations.[23] The production of aneuploid progeny by nonhomologous pairing is well documented in Drosophila melanogaster trisomies.[33]

The existence of mutants which cause aberrant chromosome behavior

leading to mosaicism and aneuploid progeny is well established in a number of organisms (for recent reviews, see 27 and 34). Such mutants have been frequently invoked to account for families with multiple aneuploids.[35,36] Baker et al.[27] have discussed four lines of evidence for cell division mutants in humans. The present case has elements of three of these. 1) Nonrandom clustering of chromosomal abnormalities within kindreds. It has been stressed that this family involves trisomies in mother and son. 2) Heritable changes in morphology of specific regions of mitotic chromosomes. Five metaphase spreads from the mother's cultured leucocytes had an extra X chromosome with a distinctive elongation and twisting in the centromeric region of the short arm (discussed in section IIB above). Thus it is mitotically heritable, though we were unable to examine cells of other relatives to determine whether it is transmitted from indiviudal to individual. 3) Gene-controlled disorders associated with mitotic chromosome instability and/or defective DNA repair processes. Mitotic instability of the extra X chromosome is indicated by the rings and fragments which were found in the proposita's cultured leucocytes. We have no evidence to determine whether this is controlled by a gene or is a property of the chromosome itself. 4) Meiotic anomalies in sterile males. This family offers no indication of fertility problems in males. However, among the sibling females in generation II (fig. 5), the infertility of the proposita (II-18) has been discussed, II-8 has had two miscarriages, and II-16 is married but has elected not to have children (behavioral infertility?). Gametogenesis has not been studied in any of these

individuals.

The simplest explanation for this family in terms of a cell division mutant would be that the proposita has a mutant which interferes with normal chromosomal behavior during mitosis to produce mosaicism in her cells and during meiosis to produce aneuploidy in her offspring. Mutants which cause nondisjunction in both mitosis and meiosis are known in Drosophila melanogaster.[37,38]

Finally, we offer a practical caveat. Had chromosome analysis been carried out as part of the infertility study of this woman, genetic counseling might have led her to avoid the trauma of bearing a trisomy 13 child. Infertility patients should be screened with dermatoglyphics, buccal smears and chromosome studies. If these analyses indicate high risk, any pregnancies can be monitored with amniocentesis.

V. Summary

This is the first reported family in which both trisomy X and trisomy 13 have occurred. A 31 year old white female with a history of ovarian dysfunction and infertility delivered a male infant with trisomy 13. Her cultured leucocytes were mosaic for trisomy X. The extra X chromosome was morphologically distinct in several cells. Other cells contained rings and fragments. Dermatoglyphic analysis showed a decreased total ridge count with five loops, three pocket whorls, one spiral whorl, and one double loop.

The nature of trisomy X and trisomy 13 are discussed with particular emphasis on the genetic transmission. Possible mechanisms to explain the two trisomies in this family are evaluated. It is suggested that infertility analysis should include evaluation of the risk of birth defects.

Acknowledgements

We would like to thank Ms. Virginia Frost for technical assistance.

References

1. Jacobs, P.A., Baikie, A.G., Court Brown, W.M., MacGregor, T.M., Maclean, N., and Harnden, D.G.: Evidence for the existence of the human "super female". Lancet, II: 423-425, (1959).

2. Jacobs, P.A., Harnden, D.G., Court Brown, W.M., Goldstein, J., Close, H.G., MacGregor, T.M., Maclean, N., and Strong, J.A.: Abnormalities involving the X chromosome in women. Lancet, I: 1213-1216, (1960).

3. Barr, M.L., Sergovich, F.R., Carr, D.H., and Shaver, E.L.: The triplo-X female: an appraisal based on a study of 12 cases and a review of the literature. Canad. Med. Ass. J., 101: 247-258, (1969).

4. Levine, H.: Clinical Cytogenetics. Little, Brown and Company, Boston (1971).

5. Daly, R.F., Patau, K., Therman, E., and Sarto, G.E.: Structure and Barr body formation of an Xp+ chromosome with two inactivation centers. Am. J. Hum. Genet., 29: 83-93, (1977).

6. Hamerton, J.L.: Human Cytogenetics. Vol. II. Clinical Cytogenetics. Academic Press, New York (1971).

7. Magenis, E. and Hecht, F.: Chromosome thirteen trisomy syndrome. In: Bergsma, D. (ED.), Birth Defects, Atlas and Compendium, 251-252 Williams and Wilkins, Baltimore (1973).

8. Hecht, F.: Chromosome eighteen trisomy syndrome. In: Bergsma, D. (Ed.), Birth Defects, Atlas and Compendium, 246-247. Williams and Wilkins, Baltimore (1973).

9. Miller, O.J.: Chromosome twenty-one trisomy syndrome. In: Bergsma, D. (Ed.), Birth Defects, Atlas and Compendium, 252-253. Williams and Wilkins, Baltimore (1973).

10. Hook, E.B. and Hamerton, J.L.: The frequency of chromosome abnormalities detected in consecutive newborn studies-differences between studies-results by sex and by severity of phenotypic involvement. In: Hook, E.B. and Porter, I.H. (Eds.), Population Cytogenetics, 63-79. Academic Press, New York (1977).

11. Carr, D.H. and Gedeon, M.: Population cytogenetics of human abortuses. In: Hook, E.B. and Porter, I.H. (Eds.), Population Cytogenetics, 1-9. Academic Press, New York (1977).

12. Jacobs, P.A.: Epidemiology of chromosome abnormalities in man. Am. J. of Epidemiol., 103: 180-191, (1977).

13. Taylor, M.B., Juberg, R.C., Jones, B., and Johnson, W.A.: Chromosomal variability in the D_1 trisomy syndrome. Amer. J. Dis. Child., 120: 374-381, (1970).

14. Taylor, A.I.: Autosomal trisomy syndromes: A detailed study of 27 cases of Edwards' syndrome and 27 cases of Patau's syndrome. J. Med. Genet., 5: 227-252, (1968).

15. Magenis, E.R., Hecht, F., and Milham, S.: Trisomy 13 (D_1) syndrome: Studies on parental age, sex ratio, and survival. J. Pediat., 73: 222-228, (1968).

16. Penrose, L.S.: Finger-print pattern and the sex chromosomes. Lancet, I: 298-300, (1967).

17. Holt, S.B.: The Genetics of Dermal Ridges. Charles C. Thomas, Springfield, Illinois (1968).

18. Lindsley, D.L. and Grell, E.H.: Genetic Variations of Drosophila melanogaster. Carnegie Inst. Washington Publ. 627 (1968).

19. Sturtevant, A.H.: Preferential segregation of the fourth chromosome in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A., 20: 515-518, (1934).

20. Sturtevant, A.H.: Preferential segregation in triplo-1V females of Drosophilia melanogaster. Genetics, 21: 444-446, (1936).

21. Khush, G.S.: Cytogenetics of Aneuploids. Academic Press, New York (1973).

22. Carr, D.H.: Chromosomal abnormalities in clinical medicine. In: Steinberg, A.G. and Bearn, A.G. (Eds.), Progress in Medical Genetics: VI, 1-61. Grune and Stratton, New York (1969).

23. Mikkelsen, M.: Down's syndrome. Current stage of cytogenetic research. Humangenetik, 12: 1-28, (1971).

24. Vandevelde-Staguet, M.F., Breynaert, R., Walbaum, R., Saint-Aubert, P., Farriaux, J.P., and Fontaine, G.: La descendance des meres trisomiques 21 (A propos d'une observation). J. Genet. Hum., 21: 187-206, (1973).

25. Hsu, L.Y.F., Gertner, M., Leiter, E., Hirschhorn, K.: Paternal trisomy 21 mosaicism and Down's syndrome. Am. J. Hum. Genet. 23: 592-601, (1971).

26. Hecht, F., Bryant, J.S., Gruber, D., and Townes, P.L.: The nonrandomness of chromosomal abnormalities. New England J. Med. 271: 1081-1086, (1964).

27. Baker, B.S., Carpenter, A.T., Esposito, M.S., Esposito, R.E., and Sandler, L.: The genetic control of meiosis. In: Roman, H.L., Campbell, A., and Sandler, L.M. (Eds.), Annual Review of Genetics. Vol. 10, 53-134. Annual Reviews, Inc., Palo Alto, California, (1976).

28. Sutherland, G.R., Fitzgerald, M.G., and Danks, D.M.: Difficulty in showing mosaicism in the mother of three mongols. Arch. of Dis. Child., 47: 970-971, (1972).

29. Hamerton, J.L., Ginnelli, F., and Polani, P.E.: Cytogenetics of Down's syndrome (mongolism) I. Data on a consecutive series of patients referred for genetic counselling and diagnosis. Cytogenetics, 4: 171-185, (1965).

30. Hecht, F.B., Neivaard, J.E., Duncanson, N., Miller, J.R., Higgins, J.V., Kimberling, W.J., Walker, F.A., Smith, G.S., Thuline, H.C. and Tischler, B.: Double aneuploidy: the frequency of XXY in males with Down's syndrome. Am. J. Hum. Genet., 21: 352-359, (1969).

31. Baikie, A.G., Garson, O.M., and Birrell, R.G.: Mosaicism for trisomy 17-18 and trisomy 13-15 in man. Nature, 207: 1419-1420, (1965).

32. Alberman, E., Elliot, M., Creasy, M., et al.: Previous reproductive history in mothers presenting with spontaneous abortions. Br. J. Obstet. Gynaecol., 82: 366-373, (1975).

33. Sandler, L. and Novitski, E.: Evidence for genetic homology between chromosomes I and IV in Drosophila melanogaster, with a proposed explanation for the crowding effect in triploids. Genetics, 41: 189-193, (1956).

34. Baker, B.S. and Hall, J.C.: Meiotic mutants: genic control of meiotic recombination and chromosome segregation. In: Ashburner, M. and Novitski, E. (Eds.), The Genetics and Biology of Drosophila. Vol. Ia, 352-434. Academic Press, New York (1976).

35. Zellweger, H. and Abbo, G.: Familial mosaicism attributable to a new gene. Lancet, I: 455-457, (1965).

36. Hsu, L.Y.F., Hirschhorn, K., Goldstein, A., Barcinski, M.A.: Familial chromosomal mosaicism, genetic aspects. Am. J. Hum. Genet., 33: 343-349, (1970).

37. Davis, D.G.: Chromosome behavior under the influence of claret-nondisjunctional in Drosophila melanogaster. Genetics, 61: 577-594, (1969).

38. Davis, B.K.: Genetic analysis of a meiotic mutant resulting in precocious sister-centromere separation in Drosophila melanogaster. Molec. Gen. Genet., 113: 251-272, (1971).

Figure 11: Marker X chromosome from five different metaphase plates from the proposita's leucocyte culture. See Appendix A for details.

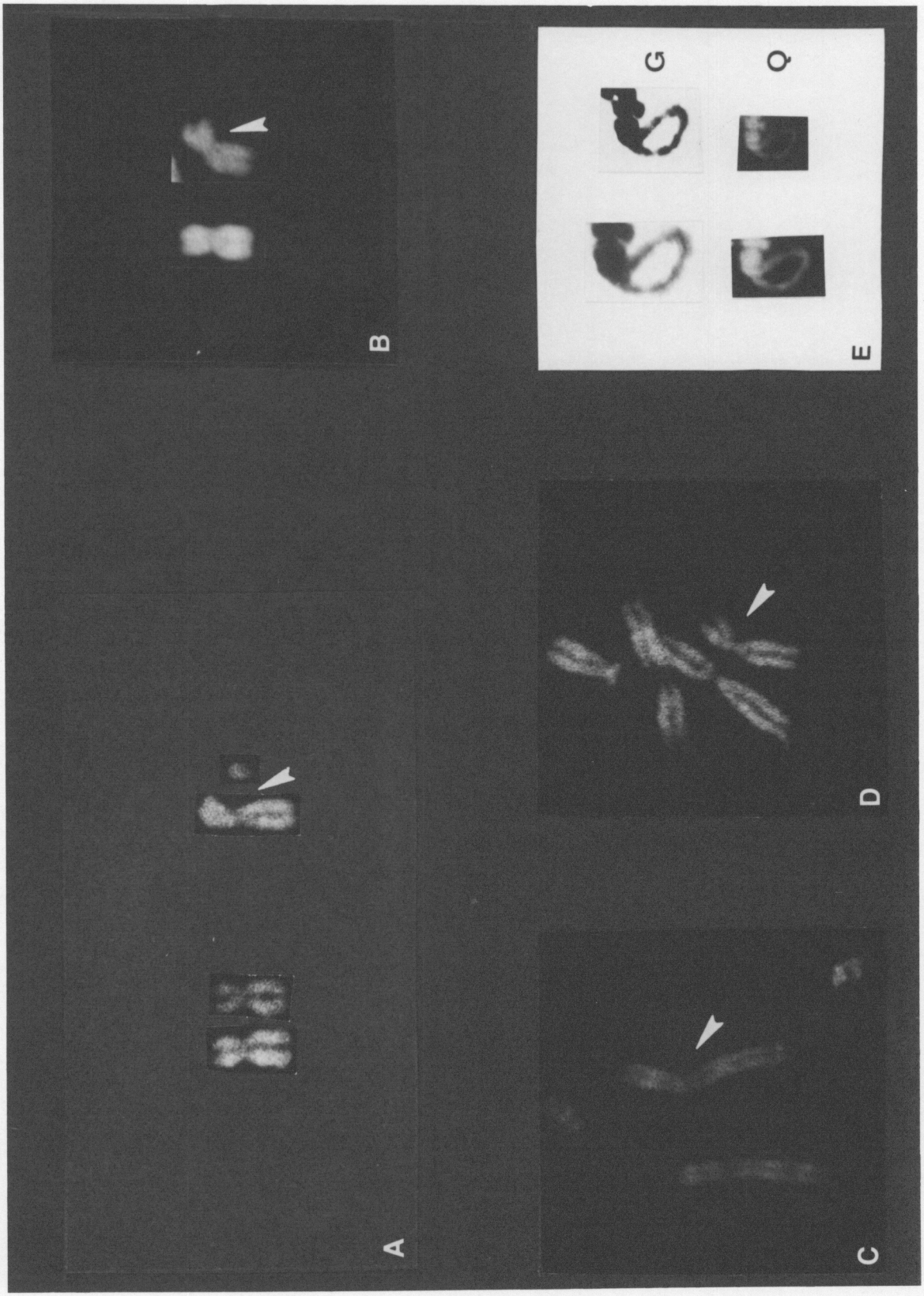


Figure 11

Figure 12: Extra chromosomes (or breakage products) recovered from five different metaphase plates. See Appendix A for details.

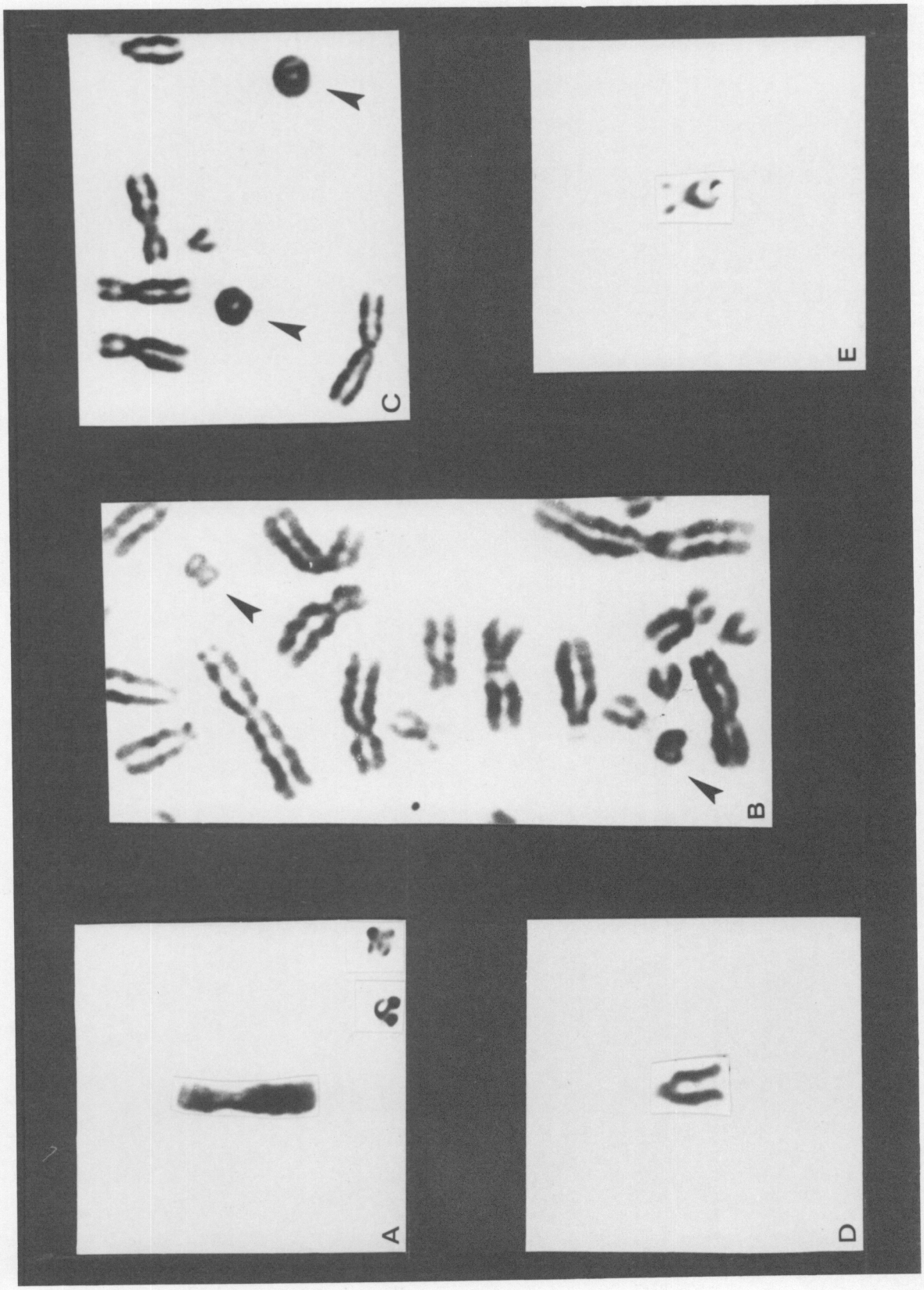


Figure 12

Appendix A

Here I describe in detail the photomicrographs from ten different metaphase plates of the proposita's leucocyte in Figure 11 and 12. For reference see page 54.

I. Figure 11

A. A Q-banded marker X chromosome is shown with a fragment and two normal X chromosomes. Note the twisting of the short arm, and the uncoiling of the long arm near the centromeric region.

B. A Q-banded marker X chromosome is indicated by the arrow along with a normal X chromosome.

C. A Q-banded marker X chromosome is indicated by the arrow. The small fragment in the lower right-hand corner shows a banding pattern similar to the distal end of the long arm of the X chromosome.

D. A Q-banded marker X chromosome is shown.

E. Four pictures (two G-banded and two Q-banded) of a marker X chromosome are shown. Note the long arm of the X chromosome is completely uncoiled forming a giant loop, joining at the center and away from the centromere. The Q-banded picture in the lower right-hand corner shows the characteristic bright band of an X chromosome in the short arm.

II. Figure 12

A. An extra chromosome, possibly an X, is shown with two fragments.

B. The arrows point to two small fragments slightly smaller than G-group chromosomes.

C. Two ring chromosomes are indicated by arrows.

Appendix A (continued)

Figure 12

D. An extra chromosome is shown, comparable in size to D-group chromosomes.

E. An extra chromosome is shown, comparable in size to E-group chromosomes.

CHAPTER IV

ABSENCE OF DERMAL RIDGES AND MOSAICISM FOR SEX CHROMOSOMES

Ranjan Y. Goradia

Brian K. Davis

Rolando J. De Leon

The following paper was presented at the Birth Defects Conference in Memphis, Tennessee held in June 1977.

I. Dermatoglyphics and Birth Defects

While the inheritance of ridge patterns is not well understood, it is clear that it is polygenic. The environmental influence is strong and a large array of genetic and environmental changes alter the ridge pattern. Both of these features are characteristic of polygenic inheritance. The best evidence for the interaction between the genes and the environment comes from studies of twins. Comparisons of total ridge count for monozygotic and dizygotic twin pairs showed much higher concordance for monozygotic twin pairs, indicating the importance of heredity.[1,2,3,4] However, the fact that dermatoglyphic patterns of monozygotic twins do differ, shows the importance of environmental influence. The environment of importance must be the uterine environment during the tenth to twenty-eighth weeks of fetal development when the ridges are developing.[5,6,7] Recently, Reed et al.[8] have compared monozygotic twins to demonstrate the interaction of genes and environment for a large number of dermatoglyphic variables.

Characteristic dermatoglyphic patterns have been demonstrated for both chromosomal aneuploids and chromosomal rearrangements (for reviews, see 7, 9, 10). This was demonstrated for trisomy 21 or Down's Syndrome by Cummins[11,12] long before the chromosomal basis was shown by Lejeune et al.[13] Subsequently, characteristic patterns were determined for autosomal trisomies (18, 13 and 8 mosaics) and for sex chromosome aneuploids (Turner syndrome and Klinefelter syndrome). An inverse relationship between total finger ridge count and a number of sex chromosomes has been demonstrated[7,14,15] and a developmental model to explain it has been

proposed.[15] Among chromosomal rearrangements, dermatoglyphic patterns have been established for Cri du Chat Syndrome (5p-), Wolf-Hirschhorn Syndrome (4p-), Chromosome 18p- Syndrome, Chromosome 18q- Syndrome, and Chromosome 18r- Syndrome.

Clinical disorders with associated abnormal dermatoglyphic patterns, which do not result from chromosomal abnormalities have been thoroughly reviewed.[7,10,16] There is a strong correspondence between disorders which affect the development of the hands and feet and those with abnormal dermatoglyphics. The etiology of these disorders includes a number of factors, such as single gene mutations, the drug Thalidomide, and infections such as rubella and cytomegalovirus infection.

Alterations in dermatoglyphic patterns from chromosomal and non-chromosomal causes involve either quantitative changes in normal patterns or qualitative changes which do not normally occur. The former includes changes in frequencies and/or positions of loops, arches and whorls, of ridges, of flexion creases, and of triradii. Qualitative changes, following the terminology of David,[17] are "ridges-off-the-end", ridge hypoplasia, ridge dissociation, and ridge aplasia, conditions, which are not normally found.

Absence of dermal ridges, also called ridge aplasia, absence of fingerprints, and ridgeless patterns, is the most extreme abnormality of ridges. It has been reported in sporadic cases,[17,18,19] in families,[20,21,22,23] and in some cases of trisomy 18 (summarized in 7). The most extensive study was that of Baird,[22,23] who identified sixteen affected individuals in four generations in an Irish-American kindred.

The trait was interpreted to be an autosomal dominant. No chromosomal abnormalities were detected in leucocyte cultures from two affected individuals. All of the sixteen demonstrated the absence of dermal ridges on fingers, toes, palms, and soles, but two had some areas of ridged skin on the hands. Transient congential milia were also found in all sixteen. Three other abnormalities, bilateral partial flexion contractures of the fingers, bilateral partial flexion contractures of the toes, were quite variable within the kindred, but showed a complete consistency within each of the three sibships in the third generation. Sweating was virtually absent in the areas with aplasia. Histological study of a skin biopsy from an affected region showed some sweat glands but the ducts did not penetrate to the epidermal surface. These secondary characteristics are not always associated with the absence of dermal ridges.

The family, reported here, has absence of dermal ridges with no associated ectodermal abnormalities. Affected individuals do have high fetal wastage and mosaicism for a deleted X chromosome.

II. Family Study

A. Family History

The propositus is a twenty year old, white male. He is presently an undergraduate student at this institution. He brought himself to our attention while taking an introductory genetics course. Physical appearance is normal with the exception that the surfaces of the hands and feet which normally have ridged skin, uniformly lacked ridged skin. There are no associated abnormalities of the skin, hair or nails, nor were they present at an earlier age.

The pedigree (fig. 1) shows that one individual has had the trait in each generation for four generations. The mode of inheritance appears to be as an autosomal dominant, but an alternative mechanism will be proposed later. A second feature which is associated with affected individuals is high fetal wastage. I-1 and I-2 produced seventeen pregnancies which miscarried or ended in death at the time of birth. Two other pregnancies produced viable offspring, though II-1 died of tetanus at the age of seven after having her ears pierced for earrings. II-7 and II-8 produced one miscarriage and four viable offspring and III-8 and III-9 produced two miscarriages and one viable offspring, the propositus. The fetal wastage is independent of the sex of the affected parent.

The summed progeny from the three couples in the pedigree in which one parent is normal and one lacks ridge patterns (I-1 and I-2, II-7 and II-8, III-8 and III-9) are compared with the summed progeny from

Figure 1: Pedigree of a family with absence of dermal ridges. The propositus is indicated by an arrow.

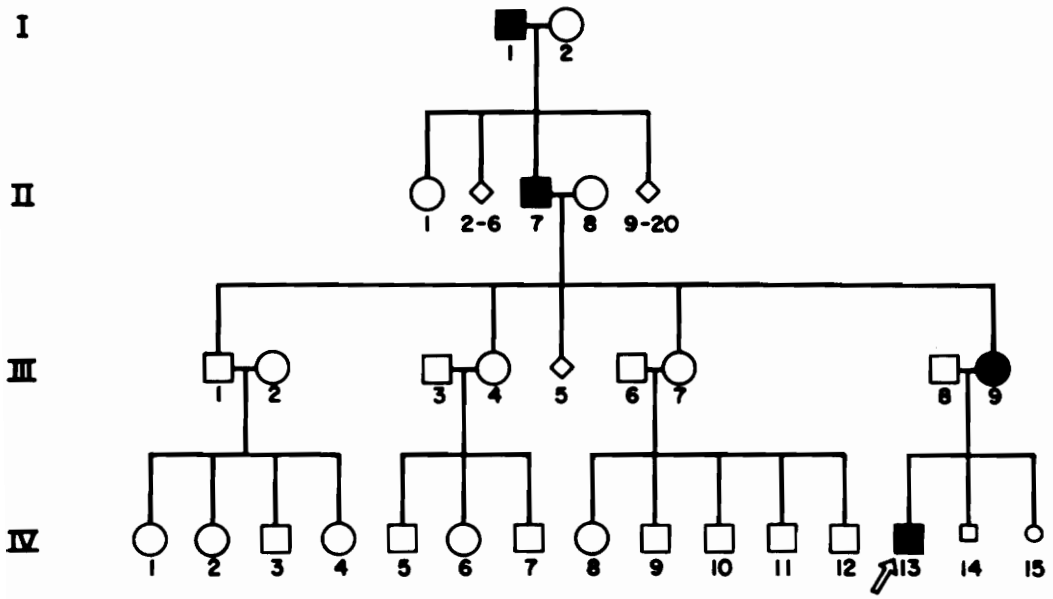


Figure 1

the three couples in which both parents have normal ridge patterns (III-1 and III-2, III-3 and III-4, III-6 and III-7) in Table 3. Although the numbers are small, several observations can be made. Two normal parents do not have affected children, while couples with an affected parent have about one half affected children. The sex ratio is about 1:1 in both groups. The relative excess of affected males over affected females is probably a chance result. The three normal couples have averaged four viable offspring (12 total children/3 couples) with no miscarriages. The three couples with an affected parent have averaged 2.3 viable offspring (7/3), but nine pregnancies (27/3).

The great-grandparents of the propositus (I-1 and I-2) came from French and Spanish families, respectively, which immigrated to Cuba. It is almost certain that they were not consanguinous, nor is there any evidence of consanguinity for any of the couples.

In 1957, the popular Cuban magazine, "Carteles", published an article on the family, including photographs of three generations of affected individuals (II-7, III-9, and IV-13).[24] There were also statements by II-7 describing the absence of ridge patterns in his father (I-1). Reference was made to an account of the family in the newspaper, "El Mundo", of San Juan, Puerto Rico.[25]

The grandfather of the propositus (II-7), was the only child of nineteen pregnancies who survived to adulthood. Other members of the family report that he had mild diabetes not requiring insulin, and that a gangrenous leg was amputated shortly before his death by heart attack at age 58.

Table 3

Reproduction in the Three Couples in which One Parent Lacks Ridge Patterns and in the Three Couples in which Both Parents are Normal

	One Affected Parent	Two Normal Parents
Miscarriages	20	0
Viable Offspring		
Affected		
Male	2	0
Female	1	0
Normal		
Male	1	7
Female	3	5
Total Offspring		
Male	3	7
Female	4	5
Both	7	12

The mother of the propositus (III-9) has deafness in one ear and has had a bilateral ovariectomy. Her present health is good. There are no abnormalities of the skin, hair or nails other than the ridge aplasia. General physical appearance is normal.

To date none of the thirteen individuals in generation IV has reproduced.

B. Dermatoglyphics

The detailed appearance of an inked print from the propositus is shown in the magnification of one area (fig. 2). There is a complete lack of organization into ridges. Raised epidermal fragments appear to be random in position and variable in size. Some fragments have a single sweat pore; a few have two pores; and rare fragments are large enough to have more than two. Some small areas totally lack fragments. An extensive report on the dermatoglyphics of this family including prints from affected and unaffected individuals will be published elsewhere.

C. Histology

Through the generous cooperation of the Montgomery County Hospital staff in Blacksburg, Virginia, histological analysis was performed on a skin biopsy taken from the thenar area of the left palm of IV-13. The tissues appear to be normal (fig. 3). The stratum corneum is detached from the stratum germinativum, but this is probably an artifact. This is being analyzed further. Sweat glands appear to be normal except for broken ducts at the region of detachment. Sweat pores can be seen in fig. 2 and both IV-13 and III-9 sweat on the volar surfaces of the

Figure 2: The details of an inked fingerprint of the propositus, showing no dermal ridges.

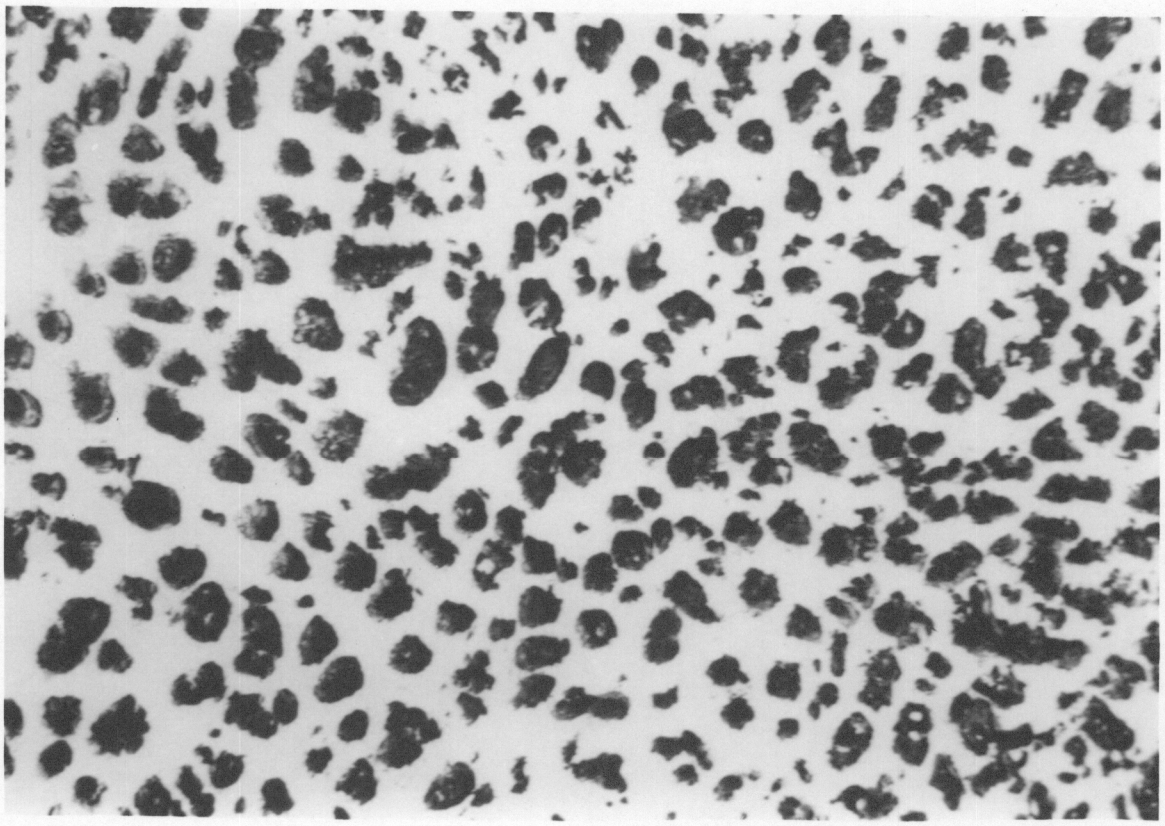


Figure 2

Figure 3: A section of a skin biopsy taken from the propositus' palm.

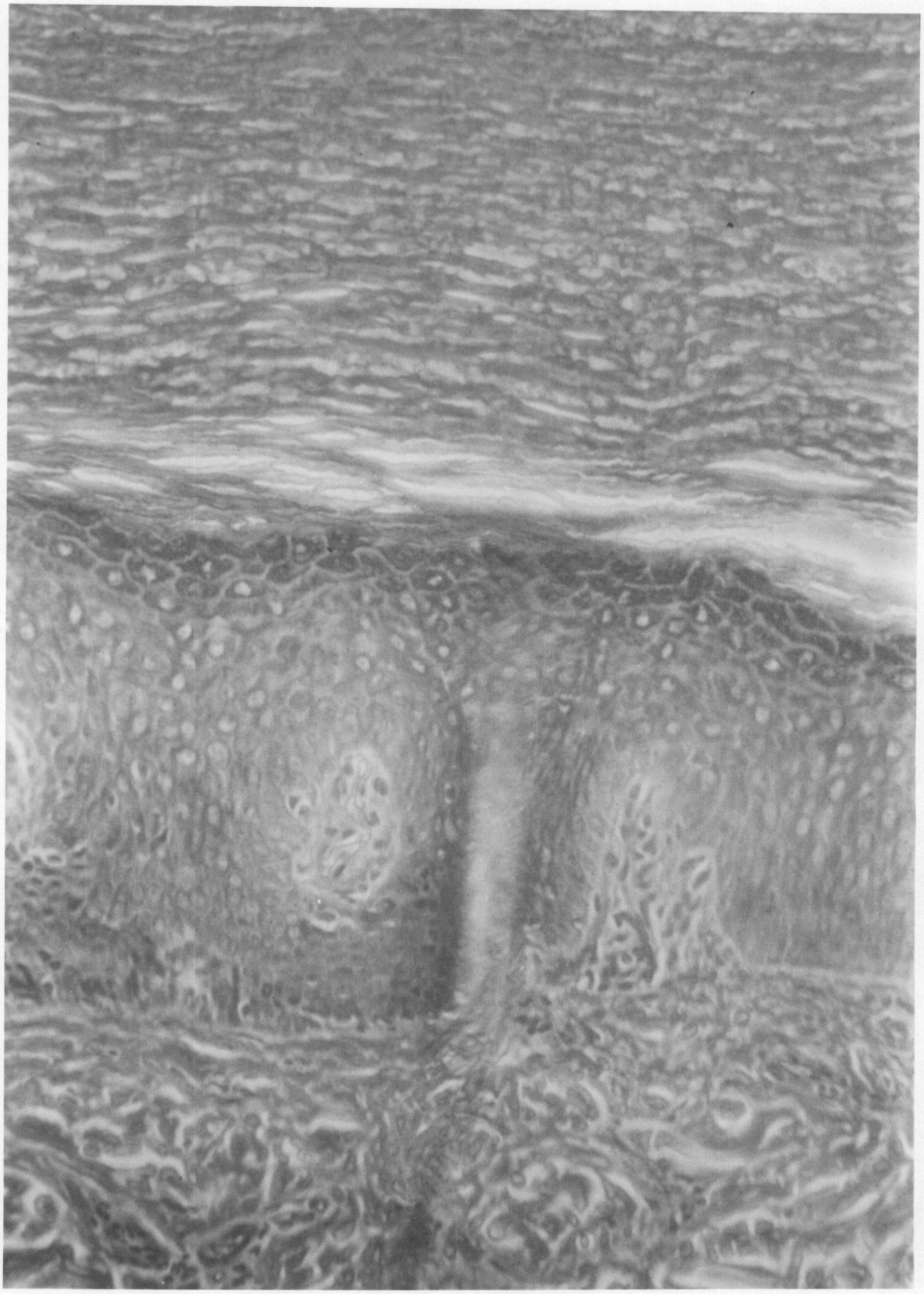


Figure 3

Figure 4: a) An enlargement of a skin biopsy section from the propositus' palm showing dermal papillae.
b) Enlargement of sweat glands.

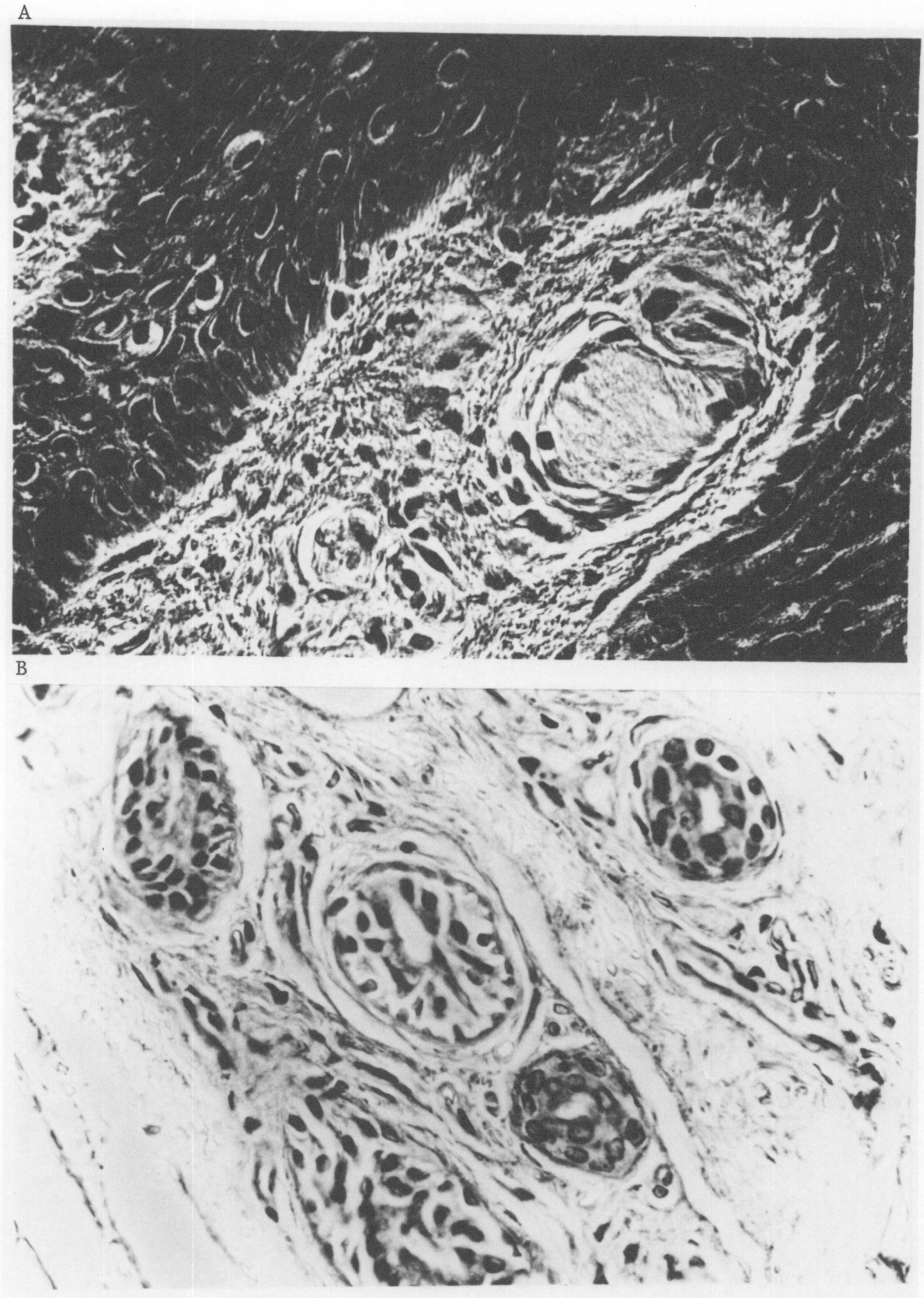


Figure 4

hands and feet. An enlargement of dermal papillae and sweat glands (fig. 4) show normal structure. The defect seems to involve only the epidermal ridges and none of the underlying structures.

D. General Aspects of the Hands

Both hands of the propositus show partial flexion (camptodactyly) of the fifth finger and possible brachydactyly (fig. 5 shows the left hand). The parameters of the hands were measured as described by Feingold and Bossert.[26] Palm length is the distance from the distal wrist flexion crease to the metacarpophalangeal flexion crease of the third or middle finger. Middle finger length is the distance from the metacarpophalangeal flexion crease to the tip of the finger. The total hand length is the distance from the distal wrist flexion crease to the tip of the middle finger or the sum of the palm length and middle finger length. Both hands of IV-13 have a palm length of 11.8 cm (0.60 of the total hand length), a middle finger length of 7.9 cm (0.40 of the total hand length) and a total hand length of 19.7 cm. For comparison, Feingold and Bossert have presented measurements based on a sample of 757 children ranging in age from birth to fourteen years.[26] These data show that palm and finger lengths are still rapidly increasing at age fourteen. While our twenty year old propositus has a palm length and a total hand length greater than 97 percent of the fourteen year olds, his middle finger length is greater than only 75 percent. His middle finger length as a fraction of total hand length is less than that of over 97 percent of fourteen year olds. This parameter is fairly constant, going from an average of 42 percent of newborns

Figure 5: The left hand of the propositus, showing absence of dermal ridge patterns.

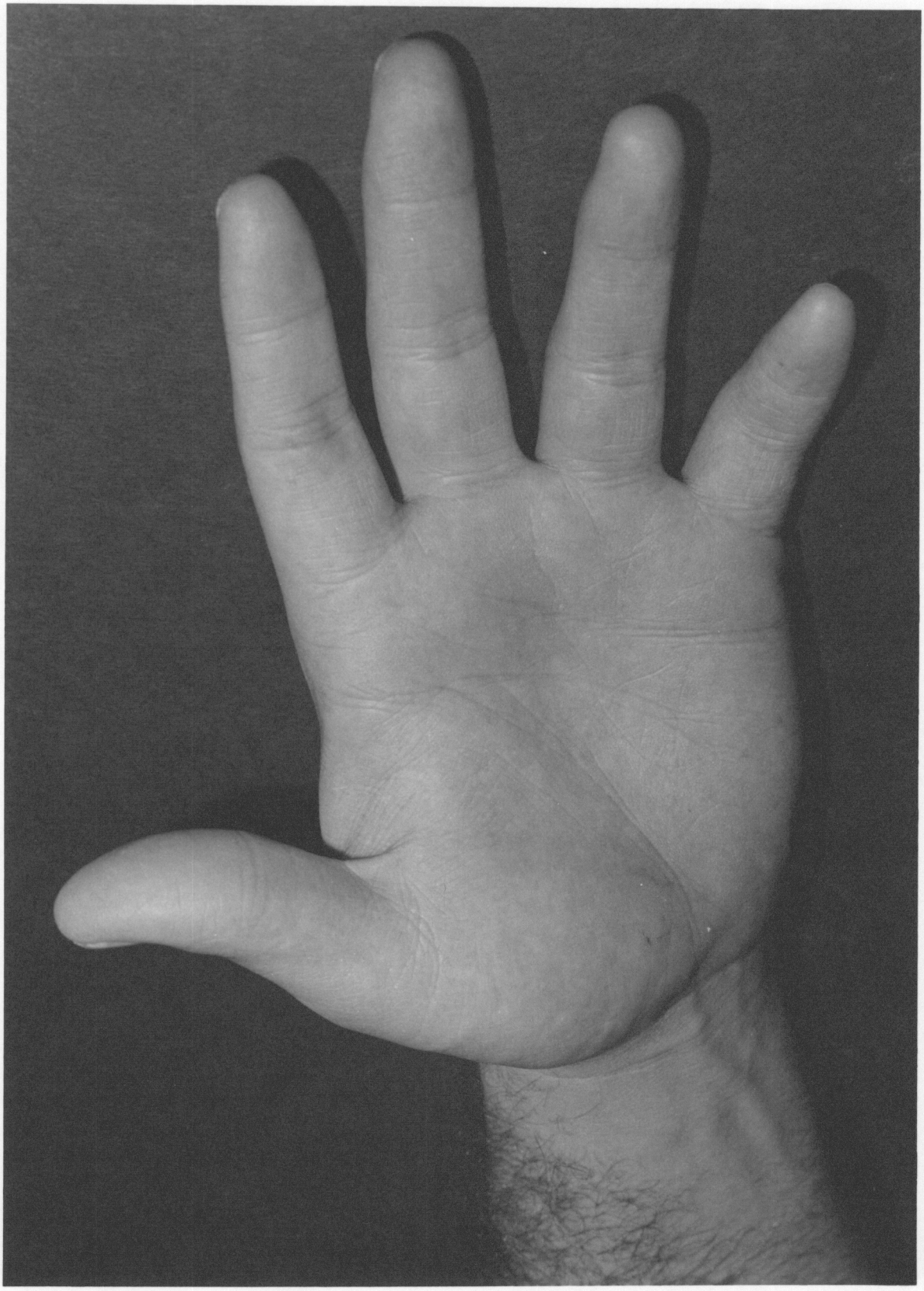


Figure 5

Figure 6: X-ray photograph of the propositus' hand.

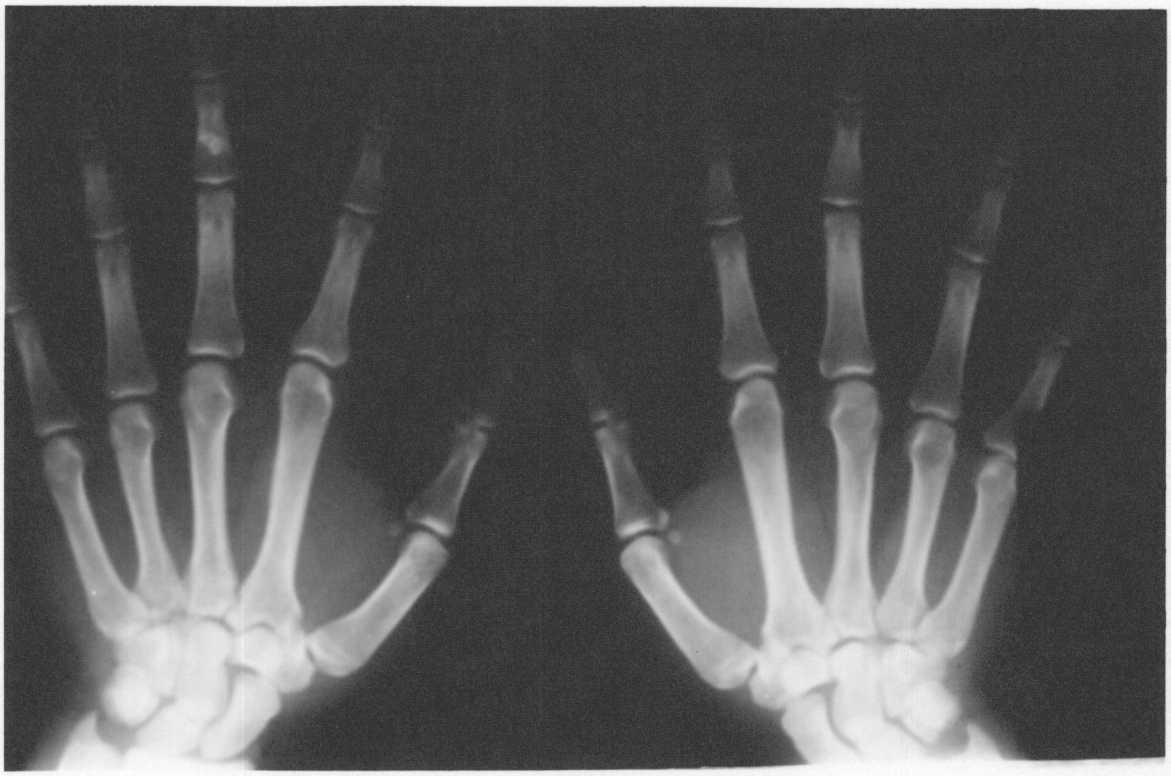


Figure 6

Figure 7: The left hand of the mother of the propositus, showing absence of dermal ridge patterns.

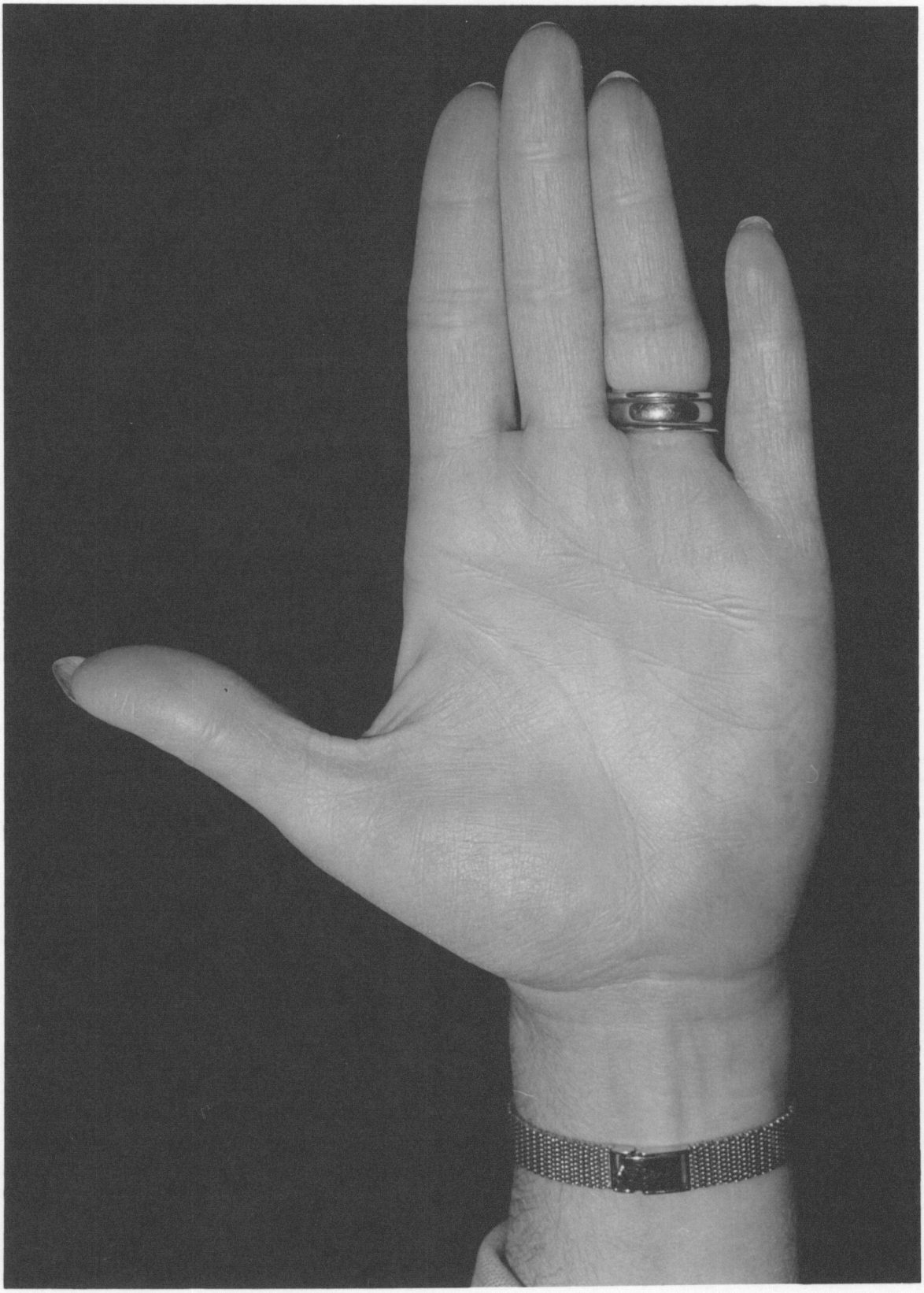


Figure 7

to 43 percent at age fourteen. The major change with age is a drastic reduction in the range of 96 percent of the population.

X-ray photographs of the hands of the propositus (fig. 6) do not indicate any of the several classes of brachydactyly.[27,28] None of the phalanges or metacarpals is shorter than normal.

The left hand of the mother of the propositus is shown in fig. 7. The proportions of her hands are similar to those of her son, but she does not have camptodactyly.

Flexion creases are normal in the hands of the propositus and his mother.

E. Chromosomes

Leucocytes from the propositus and his mother were cultured and chromosomes were banded with standard trypsin-Giemsa techniques.[29] Both individuals were shown to be mosaic for an extra X chromosome, with a complex deletion. Banding indicates that most of the short arm and a small part of the long arm are deleted. The propositus is 46,XX/47,XXdelY and the mother is 46,XY/47,XXXdel, with about eighty percent euploid cells and twenty percent aneuploid cells in each.

Typical examples of a normal X, a normal Y, and a deleted X from the propositus are shown in fig. 8 and of a normal X and deleted X from the mother are shown in fig. 9. In some cells the extra chromosome was even smaller, indicating its instability. A complete cytogenetic analysis will be presented elsewhere.

A buccal smear from the propositus was stained with orcein and examined for Barr bodies. A fraction of the cells showed a small

Figure 8: A normal X chromosome, an Xdel and a normal Y chromosome from one of the propositus' aneuploid leucocytes.

Figure 9: A normal X chromosome and Xdel from an aneuploid leucocyte of the propositus' mother.

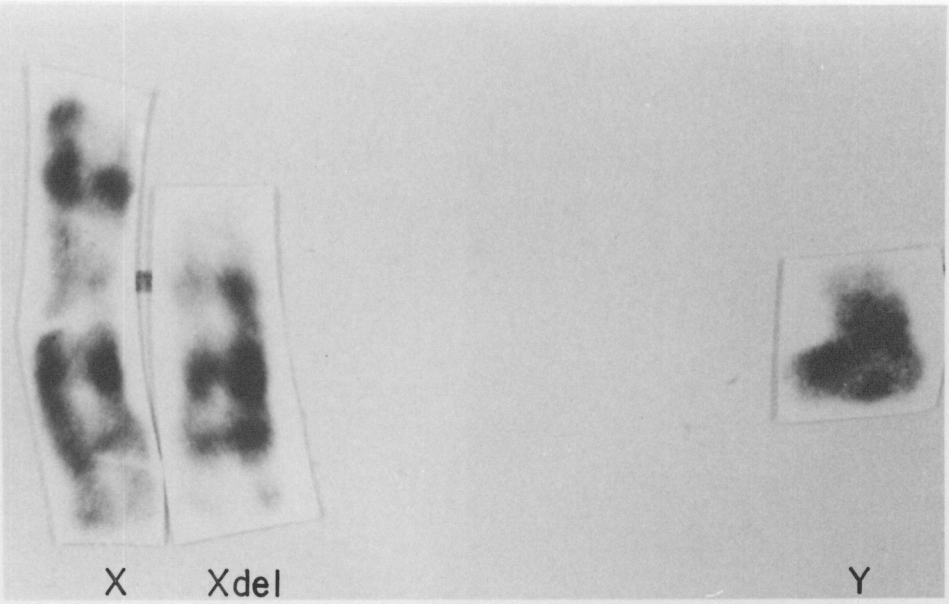


Figure 8

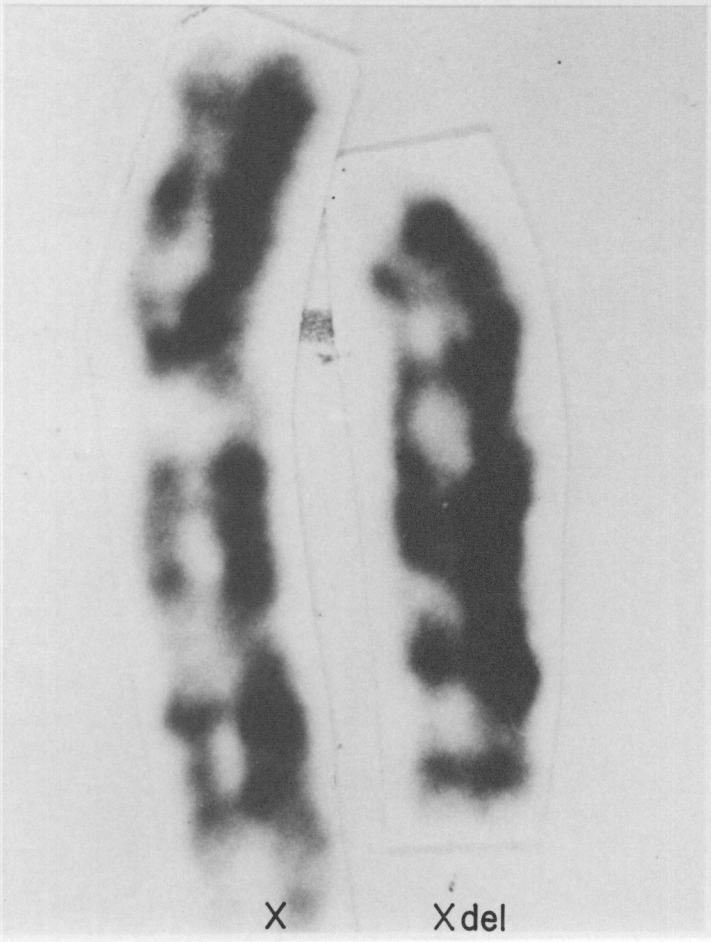


Figure 9

stained body at the periphery of the nucleus. This is presumably the inactivated Xdel.

III. Discussion

A. Interpretation of this Family

Three features characterize affected individuals in this family: 1) ridge aplasia, 2) frequent fetal wastage, and 3) mosaicism for an extra X chromosome, Xdel. We hypothesize that Xdel is inherited through a disomic egg or sperm so that affected individuals are initially 47,XXXdel or 47,XXdelY zygotes. The mitotic instability of Xdel, evidenced by the cells which have even smaller extra chromosomes, is expected to lead to chromosome loss, generating euploid lines of cells and the observed mosaicism. When these individuals reproduce, they will produce some aneuploids like themselves, some normals, and some aneuploids with one normal sex chromosome and Xdel. This last group can account for the high fetal wastage. Although the extra chromosome is clearly an X, the inheritance of the traits mimics an autosomal dominant mutation.

We have reported elsewhere in these proceedings on a mother with similar mosaicism for an unstable X chromosome who produced a trisomy 13 son.[30] Although the mechanism was probably different from that causing fetal wastage in this family, it illustrates the increased risk of an abnormal conceptus for mosaic trisomic parents.

It was stressed earlier that aneuploidy is well known to affect epidermal ridge development, though the effect of sex chromosomes is rarely this extreme.[7] The epidermal ridges come late in the sequence of skin structure development, after blood vessels and nerves are present, fetal pads have degenerated, dermal ridges develop, and sweat

glands develop. The fact that all underlying structures, including dermal papillae and sweat glands, were shown by histological examination to be normal in the propositus, suggests that the developmental interruption caused by Xdel occurs in the last few steps. This corresponds to approximately the twentieth to twenty-eighth week of fetal development.[5,6,7] Cummins has pointed out an alternative mechanism in which epidermal ridges develop normally, but then degenerate.[22,31] We have no evidence to distinguish between the two mechanisms in this family.

B. Clinical Importance of this Family

The great-grandfather of the propositus discovered that he lacked ridge patterns when he accidentally spilled a bottle of ink while at work in a cement factory and found that unlike his fellow workers, his fingers left no print patterns.[24] The grandfather's trait was revealed when the Federal Bureau of Investigation attempted to fingerprint him along with other aliens in Puerto Rico. When the propositus was born, the family discovered that he and his mother also had the trait. It has been looked upon as a family curiosity. The possible clinical significance of the absence of ridges and the fetal wastage has never been brought to the attention of the family.

The ridge aplasia is not deleterious nor is it associated with deleterious characteristics in this family. No serious birth defects have occurred. Nonetheless, dermatoglyphic abnormalities and high fetal wastage should alert the physician to potential birth defects. In this family, the demonstration of chromosomal mosaicism is a fur-

ther danger signal. Routine dermatoglyphic examination of prospective parents, followed by chromosome analysis when indicated, could be an important means in prevention of birth defects.

IV. Summary

A variety of influences on fetal development, including gene mutations, chromosomal anomalies and environmental factors, are reflected in abnormal dermatoglyphic patterns. The most extreme abnormality is the absence of dermal ridges. This report presents a family in which some individuals completely lack ridges, have high fetal wastage, and are mosaic for an extra X chromosome with deletions in both arms. There are no associated abnormalities of the skin, hair or nails. The skin histology is normal in the affected areas. A mechanism is proposed to account for the inheritance of the extra X chromosome, and the associated phenotypes, fetal wastage and absence of ridges. Finally, it is suggested that routine examination of dermatoglyphic patterns could aid in the prevention of birth defects.

Acknowledgements

We would like to thank Drs. Miguel Langebeck and Uddhavdas F. Asrani for their help with clinical aspects of this project.

References

1. Essen-Moller, E.,: Empirische Ahnlickheitsdiagnose bei Zwillingen. Hereditas, 27: 1-50, 1941.

2. Geipel, G.: Die Gesamtanzahl der Fingerleisten als neues Merkmal zur Zwillingsdiagnose. Z. Morph. Anthrop., 39: 414-419, 1941.

3. Holt, S.B.: Genetics of dermal ridges: inheritance of total finger ridge-count. Ann. Eugen., Lond. 17: 140-161, 1952.

4. Lamy, M., Frezal, J., Grouchy, J. de, and Kelley, J.: Le nombre de dermatoglyphes dans un echantillon dejumeaux. Ann. Hum. Genet., Lond., 21: 374-396, 1957.

5. Penrose, L.S. and Ohara, P.T.: The development of the epidermal ridges. J. Med. Genet., 10: 201-208, 1973.

6. Okajima, M.: Development of dermal ridges in the fetus. J. Med. Genet., 12: 243-250, 1975.

7. Schaumann, B. and Alter, M.: Dermatoglyphics in Medical Disorders. Springer-Verlag, New York, 1976.

8. Reed, T., Sprague, F.R., Kang, K.W., Nance, W.E., and Christian, J.C.: Genetic analysis of dermatoglyphic patterns in twins. Hum. Hered. 25: 263-275, 1975.

9. Holt, S.B.: The Genetics of Dermal Ridges. Charles C. Thomas, Springfield, Illinois, 1968.

10. Holt, S.B.: The significance of dernatoglyphics. Clin. Pediatr., 12: 471-484, 1973.

11. Cummins, H.: Dermatoglyphic stigmata in mongolian idiocy. (Abst.) Anat. Rec., 64(Suppl. 3): 11, 1936.

12. Cummins, H.: Dermatoglyphic stigmata in mongoloid imbeciles. Anat. Rec., 73: 407-415, 1939.

13. Lejeune, J., Geutier, M., and Turpin, R.: Etude des chromosomes somatiques de neuf enfants mongoliens. C.R. Acad. Sci. (Paris), 248: 1721-1722.

14. Alter, M.: Is hyperploidy of sex chromosomes associated with reduced total finger ridge count? Am. J. of Hum. Genet., 17: 473-475, 1965.

15. Penrose, L.S.: Fingerprint pattern and the sex chromosomes. Lancet, I: 298-300, 1967.

16. Shiono, H. and Kadawaki, J.: Dermatoglyphics of congenital abnormalities without chromosomal aberrations. Clin. Pediatr. 14: 1003-1013, 1975.

17. David, T.J.: Congenital malformations of human dermatoglyphs. Arch. Dis. Child., 48: 191-197, 1973.

18. Anonymous: Patternless fingers still identifiable. Finger Print Ident. Mag., 37: 32, 1955.

19. Rott, H.-D.: Hauleistenstrungen bei verschiedenen Krankheiten. In: Hirsch, W. (Ed.), Hautleisten and Krankheiten, 247-268. Ernst-Reuter-Gesellschaft, Berlin, 1971.

20. Ludy, J.B.: Congenital absence of fingerprints. Arch. Dermatol. Syphil., 49: 373, 1944.

21. Basan, M.: Ektodermale Dysplasie. Fehlendes Papillarmuster, Nagelveranderungen and Vierfingerfurche. Arch. Klin. Exp. Derm., 222: 546-557, 1965.

22. Baird, H.W.: Kindred showing congenital absence of the dermal ridges (fingerprints) and associated anomalies. J. of Pediatr., 64: 621-631, 1964.

23. Baird, H.W.: Absence of fingerprints in four generations. Lancet, 2: 1250, 1968.

24. Coello, R.: Un misterio biologico. Padre, hija y nieto sin huellas digitales. Carteles, 38(29): 36-37, 1957.

25. "El Mundo" of San Juan, Puerto Rico, p. 9. November 9, 1940. Cited in Coello, R.: Un misterio biologico. Padre, hija y nieto sin huellas digitales. Carteles, 38(29): 36-37, 1957.

26. Feingold, M. and Bossert, W.H.: Normal values for selected physical parameters: an aid to syndrome delineation. Birth Defects, 10(13): 1-15, 1974.

27. Temtamy, S.A.: Brachydactyly. In: Bergsma, D. (Ed.), Birth Defects, Atlas and Compendium, 214-215. Williams and Wilkins Co., Baltimore, 1973.

28. Kelikian, H.: Congenital Deformities of the Hand and Forearm. W.B. Saunders Co., Philadelphia, 1974.

29. Arrighi, F.E. and Hsu, T.C.: Staining constitutive heterochromatin Giemsa crossbands of mammalian chromosomes. In: Yunis, J.J. (Ed.), Human Chromosome Methodology, 59-71. Academic Press, N.Y., 1974.

30. Goradia, R.Y., Davis, B.K., and Mann, J.: Sex chromosome mosaicism in a mother and trisomy 13 in her child. These proceedings.

31. Cummins, H.: Loss of ridged skin before birth. Finger Print Ident. Mag., 46: 3-7, 1965.

CONCLUSION

Although considerable progress has been made in the last twenty years, medical genetics research is still in its infancy. Our knowledge concerning the structure and behavior of genes and chromosomes and the manner in which genetic diseases are transmitted from generation to generation giving rise to various abnormalities, is very limited. The four papers presented here deal with various aspects of chromosomes and birth defects.

An attempt was made to understand the various factors responsible for banding and spiralization in human chromosomes. By varying the amount of heat, I was able to produce bands, spirals or a combination of both. Apparently, heat denaturation of chromatin makes available to the action of trypsin some chromosomal proteins which would otherwise be protected. Removal of these proteins changes the chromosome morphology from bands to spirals. If these proteins can be identified one can understand the microscopic variation in chromosome condensation and coiling.

In particular, if these proteins are non-histone structural proteins, they could provide an important gap left in the hierarchy of helices proposed by Bak et. al., (see Ref. 9 in Introduction). It may be that these proteins form crosslinks which hold the unit fiber in the highest level of coiling in the chromosome. I believe that the photomicrograph on page 17, Figure 2b, may represent the unfolding of this unit fiber. If these structural non-histone proteins are partially removed, the final folding of the unit fiber can be altered in several

ways. First, when some of the crosslinking proteins are removed, the unit fiber packing is relaxed, and as a result, the bright Q-bands should become wider and possibly brighter if DNA was more accessible to the dye. It is quite possible that the photomicrograph on page 32, Figure 1, represents such an effect.

Another speculation is that if the direction of coiling of the unit fiber is opposite in the two sister chromatids, and if the crosslinking proteins are removed, then the coils will relax in opposite direction, giving rise to asymmetric bands as observed in the photomicrograph on page 37, Figure 3.

It is also generally believed that the two sister chromatids have the same chemical composition. Unless there is some organizational difference between sister chromatids, it is difficult to explain the fact that the two chromatids move towards opposite poles during anaphase. There must be some mechanism preventing two spindle fibers from the same pole attaching to sister chromatids. The structural difference of centromeres or different helicities in sister chromatids may be the underlying factor for spindle fiber recognition. The difference of centromeres may be due to the opposite helical arrangement of sister chromatids. Therefore, I propose that organizational differences between sister chromatids are necessary to prevent frequent non-disjunction.

The third paper concerns a female with trisomy XXX mosaicism who gave birth to a child with trisomy 13. The female had been a patient

in an infertility clinic before her pregnancy. Such patients should be monitored by amniocentesis. Our investigation also suggests the possible existence of meiotic mutants in humans. In one of our metaphase plates we may have cytological evidence of an X chromosome which uncoils itself in its entire long arm due to possible failure of the inactivation mechanism. This conjecture is based upon the recent finding presented by Summitt at the 1977 Annual Meeting of Birth Defects in Memphis that the genes controlling the inactivation are located in the long arm of the X chromosome near the centromere.

Finally, the last paper deals with a case of rare occurrence -- absence of dermal ridges in four successive generations. A study of the pedigree showed high fetal wastage. A deleted X chromosome (Xdel) is apparently inherited through a disomic egg or sperm so that the affected individuals are initially 47,XXXdel or 47,XXdelY zygote. The mitotic instability results in the frequent loss of Xdel, thus generating euploid cells with the observed mosaicism. When the mosaic individuals reproduce, they are expected to have three varieties of offspring: i) aneuploids like themselves, with ridge aplasia; ii) normals; iii) aneuploids with one normal sex chromosome and one Xdel. The last group will account for the high fetal wastage. Although the extra X chromosome is clearly an Xdel, the inheritance of the trait mimics an autosomal dominant mutation.

It is hoped that the findings of our last paper will stimulate the use of dermatoglyphics in medical genetics laboratories as an effective screening device to prevent future birth defects -- most ubiquitous of all human maladies.

VITA

Ranjan Yogesh Goradia was born in Surat, India. She attended Home School in Bhavnagar, India and graduated from high school in June 1956. She received her Bachelor of Science with a chemistry major and botany minor from the University of Bombay in 1960 and her Master of Science in Genetics from the University of Oregon in 1972. During the last fourteen years, she has worked as a chemist, biochemist and medical geneticist at various locations in the United States until she joined V.P.I. & S.U. in September, 1975 as a graduate student. She is married to Yogesh N. Goradia and they have a daughter, Shree aged 15.

Ranjan Y. Goradia.

Ranjan Y. Goradia

HUMAN CHROMOSOMES: STRUCTURE, ABNORMALITIES AND BIRTH DEFECTS

by

Ranjan Y. Goradia

(ABSTRACT)

The research presented in this dissertation consists of four papers that revolve around the structure of human chromosomes and their relationship to birth defects.

A new technique is described to produce spiralization of human metaphase chromosomes. The important feature is heat followed by trypsin treatment. By varying conditions, it is possible to produce bands, spirals and intermediate states.

An investigation of human metaphase chromosomes reveals identical lateral bands in sister chromatids when stained with Quinacrine mustard or Giemsa-trypsin. A hybrid of these two methods produces banding patterns which are different in sister chromatids yet may be repeated in homologous chromatids.

A case study is presented in which a 31-year old white female with a history of ovarian dysfunction and infertility delivered a male infant with trisomy 13. Her cultured leucocytes were mosaic for trisomy X. The natures of trisomy X and trisomy 13 are discussed with particular emphasis on the genetic transmission.

In another case study of a family, it is found that some individuals who completely lack dermal ridges are mosaic for an extra X chromosome with deletions in both arms. A mechanism is proposed to account for the extra chromosome.