THE ROLE OF Q-BANDING IN THE CYTOGENETIC STUDY OF HUMAN SPONTANEOUS ABORTIONS

BY

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SCOPE AND CONTENTS:

The discovery of Q-banding made it possible to identify every member of the human chromosome complement. Spontaneous abortions known to have a better than average chance of presenting with a chromosome anomaly were selected for cytogenetic analysis. A highly successful technique for culturing chorion from these specimens was developed. Direct chromosome preparations were obtained from the coverglasses on which the fibroblasts were subcultured. The characteristics of each specimen were noted so that as the information pool grows, it may be possible to define abortion syndromes.

Fifty-nine per cent of the specimens selected were abnormal. These abnormalities included trisomies 2, 8, 14, 16 and 22, triploidy and tetraploidy. Vesicular villi, maternal age over 40 and conception coincident with maternal ingestion of contraceptives were found to be excellent forecasters of chromosome anomalies. Only one embryo, 69,XXY, in which congenital malformations could be identified was collected during the duration of this project. A possible polymorphism in a non-heterochromatic region of chromosome 17, in a 16-trisomy specimen, was noted.

Heteromorphic bands have made it possible to distinguish between the members of a homologous pair of chromosomes 3, 4, 13, 14, 15, 21 and 22. A study of such markers in a twin abortus allowed for speculation on the zygosity of the embryos. Similar polymorphisms were used to determine whether the extra set of chromosomes in two triploid abortuses was of maternal or paternal origin. Distribution of these marker chromosomes was also used to determine when in meiosis the event of abnormal development occurred. The point was stressed, that some cell-to-cell variability does occur in these heteromorphic Q-bands and that great care must be taken in distinguishing maternal and paternal marker chromosomes, before the distribution of these parental chromosomes can be used to make statements about abnormal developmental events.

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TABLE OF CONTENTS

DISCUSSION (cont'd) page

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

I. Chromosome Banding Techniques

In 1969, Caspersson et al. reported that scientists, seeking chemical bases for the transfer of differentiation information through metaphase, had been encouraged by the discovery that alkylating agents attached at the N-7 atom of guanine in DNA. This led to the hypothesis that segments of chromosomes with varying base composition could be detected because fluorescent alkylating agents would interact preferentially with guanine, producing much brighter fluorescence in areas rich in guanine and cytosine (G-C regions) than in sections where adenine and thymine residues predominated (A-T regions).

Working with metaphase chromosomes of Vicia fava, Caspersson and his colleagues (1969) were able to demonstrate the validity of this hypothesis. After treatment with quinacrine mustard (QM), all of the chromosomes showed clear, unique and reproducible patterns of cross-striations extending across both sister chromatids.

It was not long before the technique of fluorescent banding was applied to human material. Using photoelectric techniques, Caspersson et al. (1970a, 1970b) showed that each human chromosome had a unique QM banding pattern. Up until this time, human cytogeneticists had had no means of accurately identifying all members of the complement since human chromosome pairs could be very similar in arm length and centromere position to as many as seven other pairs.

Taking Caspersson's work one step further, Fleischmann et al. (1971), found that even finer analysis and better visualization of fluorescence patterns were possible by employing digitized pictures, computer processing and display on a curve-plotter. However, this sophisticated apparatus and that used by Caspersson are hardly common in a cytogenetics laboratory. The modified techniques of Lin et al. (1971) and O'Riordan et al. (1971) greatly simplified the procedure for obtaining information from fluorescent bands so that it could be used regularly in diagnostic research. These methods allowed visual analysis of the bands, thereby eliminating the need for a reflection photometer, and used the fluorochrome, quinacrine dihydrochloride (Q), which, marketed as Atebrin or Mepacrine, is more readily available and less expensive than QM.

It soon became evident that there were many ways of getting reproducible banding patterns on chromosomes. Incubation of fixed chromosomes in warm saline (Sumner et al., 1971) or buffer solution (Schnedl, 1971) before staining with Giemsa, or pretreatment with trypsin before staining with Leishman (Seabright, 1971), as well as simple staining in Giemsa with the pH adjusted to 9.0 (Patil et al., 1971) resulted in banded chromosomes. These bands were created because certain segments of the chromosome stained very darkly while adjacent areas took up very little stain. It was observed that, with 3 exceptions, the dark or positively stained G-bands were located at exactly the same places on the same chromosomes as Q-positive bands (bands which fluoresced more than adjacent bands), while the light or negatively stained G-bands corresponded to Q-negative bands (bands which fluoresced less than adjacent bands). The secondary

constrictions in the long arms of chromosomes 1 and 16 (abbreviated to 1qh and 16qh respectively), which were Q-negative, stained positively (darkly) with Giemsa. Also, the brilliant Q-band at the end of the long arm of the Y chromosome (the distal Yq band) had a variable G-band counterpart.

Using the information that anti-nucleoside antibodies react with denatured DNA, Dev et al. (1972) employed formamide denaturation and indirect immunofluorescence of an anti-adenosine antibody (anti-A) to locate the A-T regions of chromosomes. The resulting pattern of immunofluorescence turned out to be a series of bands (anti-A bands) found to be the same as Q-bands, with one deviation. The distal Yq Q-band failed to react with anti-A, suggesting to the authors that this region might be resistant to denaturation.

By decreasing the period of saline incubation from 1/2 - 1/6 of that used by Sumner et al. (1971), Dutrillaux and Lejeune (1971) produced a pattern of dark and light bands designated R-bands. R-bands were complementary to Q-bands (i.e. Q-positive segments stained negatively with Giemsa, while Q-negative segments stained positively with Giemsa) on all chromosomes except 1, 9, 16 and Y. The Q-negative 1qh, 9qh and 16qh regions stained negatively with Giemsa and the distal Yq R-band was variable.

Schreck et al. (1973) destroyed guanine residues by photo-oxidation in the presence of methylene blue. The binding of a cytosine specific antibody to exposed cytosine residues was detected by indirect immunofluorescence. The resulting anti-C bands were identical to R-bands implying that regions 1qh, 9qh and 16qh, which show almost no fluorescence after Q-staining, were also very pale after treatment with anti-C. The possibility that these regions, too, might be particularly resistant to denaturation was noted.

It is not surprising that Pardue and Gall's method for detecting mouse satellite DNA (1970) was applied to human chromosomes, for it was in experimentation with their technique that Sumner first observed G-bands. The procedure called for prolonged NaOH treatment of chromosomes followed by saline incubation and Giemsa staining and the result was C-bands, a fourth banding pattern for human chromosomes (Arrighi and Hsu, 1971; Evans et al., 1971). The constitutive heterochromatin was localized in densely staining regions at the centromere of each chromosome as well as in the distal Yq band and the satellites found on chromosomes 13, 14, 15, 21 and 22. The rest of the chromosomes remained pale and unbanded. This technique falls into a different category than those producing Q-, G- and R-bands since C-bands do not permit the identification of each individual chromosome.

Mention of band polymorphisms was delayed until this point, not because of its late discovery or relative unimportance to future cytogenetic research, but simply to avoid making the introductory discussion of banding needlessly complex.

As soon as cytogeneticists started to publish human karyotypes, it became evident that certain Q-bands were polymorphic, while G- and R-band staining methods did not clearly demonstrate these polymorphisms. It is now known that the Q-bands which appear near the centromeres of chromosomes 3, 4, 13, 14, 15, 21 and 22, as well as the distal Yq band, can vary in length and intensity, and that these variations are constant - depending on chromosome contraction, time exposed to UV light, the plane of the chromosome, and understaining or overstaining - within all cells of an individual (Paris Conference, 1971). The C-bands on chromosomes 1, 9 and 16 and the distal Yq

C-band have also been found to be present in polymorphic forms which are constant for one individual (Evans et al., 1971). All of these polymorphic markers make it possible to distinguish between normal and morphologically identical chromosomes of certain homologous pairs. This tool has an important role in studies of chromosome origin and abnormal embryonic development.

Satellite variation had been identified long before the banding techniques were developed. This morphologic variability in satellite size or density is reflected by variation in the size and staining intensity of Q-, G-, R- and C-bands (Paris Conference, 1971). However, as was noted with the conventional carbol fuchsin or orcein chromosome stains, satellites do not appear in all cells since there is cell-to-cell variability in the staining of the short arms of the acrocentric chromosomes. Also, the satellites may be bent under the rest of the chromosome so that they escape notice. Thus, the presence of very bright satellites in all cells that show satellites can be used as an accurate means of tracing the origin of the chromosome with brightly fluorescing satellites, while the presence or absence of Q-negative satellites is a marker which requires careful interpretation.

As a result of these rapid and significant advances in the field of human cytogenetics, the Fourth International Conference on Standardization in Human Cytogenetics was held in Paris in September, 1971.

At this time, agreement was reached that a band would be defined as, "that part of a chromosome which was clearly distinguishable from its adjacent segments by appearing darker or lighter with the Q-, G-, R- or C-staining methods." Thus, chromosomes would be

visualized as consisting of a continuous series of light and dark bands and, by definition, there would be no "interbands" (Paris Conference, 1971).

The description of the standard human karyotype, based on the fluorescent staining pattern, appears below in excerpts taken directly from the publication stating the decisions made as a result of the Paris Conference.

"In the description that follows only major fluorescent bands will be referred to, even though in some cells these may appear to consist of several smaller bands. Faintly fluorescing bands are not referred to except when they are of special significance; generally, it may be assumed that they separate the major fluorescent bands or are located at the ends of the chromosome arms.

In the description, diagnostic features indicated by "A" are those seen in fluorescent metaphases of fair technical quality whereas those indicated by "B" are usually visible only in cells of good quality. When these details are not included in the text, the banding pattern is identical to that described under "A". Features which may vary in fluorescent intensity or length or both between individuals and between homologs are indicated by "C". The terms "distal" and "proximal" refer to the position of a band in respect to the centromere; "centric" means the area occupied by the centromere.

Some mitoses show considerable nonuniformity in that the homologous chromosomes may differ greatly in overall fluorescence and relative length. Identification must be based, therefore, on the fluorescent banding patterns of the individual chromosome rather than

on its overall intensity. However, intensity may serve as a secondary criterion, if due allowance is made for nonuniformity. The following terms will be used to indicate the approximate intensity of fluorescence:

negative	no or almost no fluorescence
pale	as on distal 1p
medium	as the two broad bands on 9q
intense	as the distal half of 15q
brilliant	as on distal Yq

No. 1 — The long arm is that previously defined as the arms with a proximal secondary constriction.

A p: Distal, pale segment grading to a proximal, medium fluorescent segment.

q: Central, intense band. Proximal, negative secondary constriction.

B p: Proximal, medium fluorescent segment; divisible into two bands.

q: Five medium fluorescent bands; central one most prominent.

C q: Negative secondary constriction variable in length.

No. 2

A Medium fluorescence along the whole length.

B p: Four medium fluorescent bands; two central ones often appear as a single segment.

q: Two central bands, sometimes accompanied by another two, all of medium fluorescence. Additional bands can be seen sometimes.

No. 3

A Single pale band in center of each arm separating medium fluorescent segments. Distal, medium fluorescent segment; longer in q than in p.

B Single pale band at end of each arm; longer in p than in q.

C q: Proximal band of variable fluorescence.

No. 4

A Medium fluorescence along the whole length.

B p: Single central, medium fluorescent band.

q: Proximal, intense band. Distal, pale band.

C Intense centric band.

No. 5

A q: Central, long, medium fluorescent segment. Distal, pale segment.

B p: Single medium fluorescent band; shorter and brighter than on 4p.

q: Distal, pale segment; divisible into a proximal, pale band and a distal, medium one.

No. 6

A p: Central, pale band separating medium fluorescent segments.

q: Medium fluorescence along entire length.

B q: Four medium fluorescent bands.

No. 7

A p: Distal, short, medium fluorescent band.

q: Two central, intense bands. Distal, medium fluorescent band.

p: Proximal, medium fluorescent band.

No. 8

A Medium fluorescence along the whole length; q brighter than p.

B p: Two evenly spaced, medium fluorescent bands.

q: Two medium fluorescent bands in distal half; brighter than those on p.

No. 9

A q: Proximal, negative segment corresponding to the secondary constriction. Two evenly spaced, medium fluorescent bands distal to the negative segment.

B p: Central, medium fluorescent band.

C q: Proximal, negative band (secondary constriction) variable in length.

No. 10

A p: Medium fluorescence.

q: Three evenly spaced bands; the most proximal one intense and the others medium in fluorescence.

No. 11

A p: Medium fluorescence; longer than 12p.

q: Short, medium fluorescent band adjacent to the centromere; separated by a negative band from a more distal, medium fluorescent segment.

No. 12

p: Medium fluorescence; shorter than 11p.

q: Medium fluorescent band adjacent to the centromere; separated by a short, negative band from a more distal, medium fluorescent segment. Distal segment longer than that of 11q.

No. 13

A q: Distal half intense.

B q: Distal half intense; divisible into two bands.

C p: Satellites and/or short arms with variable fluorescence.

q: Proximal, intense band.

No. 14

A q: Proximal half intense. Distal half pale; medium fluorescent band close to the distal end.

C p: Satellites and/or short arms with variable fluorescence.

No. 15

A q: Proximal half medium in fluorescence. Distal half pale; less fluorescent than either 13q or 14q.

C p: Satellites and/or short arms with variable fluorescence.

No. 16

A p: Medium fluorescence, less fluorescent than q.

q: Proximal, negative segment corresponding to the secondary constriction. Distal to it, a medium fluorescent segment.

C q: Negative secondary constriction variable in length.

No. 17

A p: Overall pale fluorescence.

q: Two segments of similar length; proximal one pale and distal one medium in fluorescence.

q: Narrow negative band separating proximal and distal segments.

No. 18

A p: Overall medium fluorescence.

q: Medium fluorescence; brighter than p.

B q: Two bands of medium intensity; proximal one longer and brighter than distal one.

No. 19

A Most weakly fluorescent chromosome in the karyotype. Short, proximal fluorescent bands on both arms; pale when compared to the whole karyotype.

B Fluorescent band longer and brighter on p than on q.

No. 20

A Overall pale fluorescence; p medium and q pale in fluorescence.

No. 21

A q: Proximal, intense segment. Distal, pale segment.

C p: Satellites and/or short arms with variable fluorescence.

No. 22

A Overall pale fluorescence.

B q: Narrow, pale band in center of arm.

C p: Satellites and/or short arms with variable fluorescence.

X

A p: Proximal, pale segment. Central, medium fluorescent band.

q: Proximal, pale segment. Distal to it, a medium fluorescent band.

B q: Three evenly spaced, medium fluorescent bands; most proximal one brightest.

Y

A p: Overall pale fluorescence.

q: Proximal segment pale. Distal segment brilliant.

C q: The brilliant fluorescent segment on the end of q may vary in length and may be subdivided into two or more bands. The normal variation in length of the chromosome is associated with variation in length of the brilliant segment" (Paris Conference, 1971).

Another product of this conference was the development of a system of chromosome landmarks and regions, which allows for accurate description of complex chromosome rearrangements and, also, for the subdivision of original bands. Expert cytogeneticists are certain that these sub-bands will be forthcoming with the refinement of the banding techniques (Paris Conference, 1971).

It is indeed disturbing that the mechanism responsible for the now widely used phenomenon of chromosome banding is not known. Many scientists have risen to this challenge. Unfortunately, the results from different laboratories have often been contradictory. The present state of the problem is outlined below.

Gamner and Evans (1971) found, with the exception of the late labelling-X and the centromeric areas of chromosomes 1, 9, 16 and 22, that patterns of late replication as shown by autoradiography correspond to Q-positive bands. This implied that chromatin in these bands was not euchromatic but heterochromatic.

The results of the indirect immunofluorescence studies previously described (Dev et al., 1972; Schreck et al., 1973) leave little doubt that there are large blocks of repetitive DNA in human

chromosomes and that it is in the repetitive A-T regions that the Q- and G-bands are formed. R-bands must then occur in the G-C regions.

In retrospect, it seems reasonable that bands should demarcate segments of repetitive DNA, since there is little cause to believe that adjacent sequences of several hundred unique structural genes (i.e. 2 "bands" of euchromatin) could vary in overall base composition to the extent suggested by the marked staining differences between adjacent Q-, G- or R-bands.

Schreck et al., (1973) sited evidence that the distribution of nucleoproteins was related to the base composition of DNA in localized areas and that non-histone proteins have different degrees of reaction to procedures such as photooxidation. As far as the mechanism of banding is concerned, this means that the non-histone proteins peculiar to A-T segments could react differently to the pre-staining treatments of the banding techniques than the other non-histone proteins associated with G-C regions. Then, possibly, in the banding technique, the acidic protein-DNA relationships are disrupted in one block of repetitive DNA, so that this segment of the chromatin can take up stain, while in an adjacent block of DNA, which is associated with different proteins, the chromatin structure remains intact and does not react with the molecules of the stain.

This line of reasoning indicates, then, that it is the relationship between DNA and non-histone protein which is the important factor in banding. Comings et al., (1973) arrived at a similar conclusion in a recent extensive study of G- and C-banding, after they eliminated the alternatives such as (1) a higher concentration

of DNA in dark G-band regions, (2) different base composition, (3) preferential renaturation of repetitious DNA and (4) selective disruption of light G-band DNA. They found that G-banding techniques, including the one with trypsin, remove very little protein and less than 9% of the DNA from chromosomes. This is further evidence in favour of reorganization rather than destruction of the chromatin as a result of the G-banding procedure. These workers then proceeded to explain C-banding as follows: since C-band heterochromatin is so resistant to rearrangement, very harsh treatment is required to produce C-bands. This treatment disrupts the rest of the chromosome so much (80% of the DNA is removed) that G-banding cannot take place.

Since Q- and G- bands are produced at the same places on metaphase chromosomes, Q-banding must be operating on a principle similar to that of G-banding. If so, a non-histone protein-DNA rearrangement may occur in the A-T regions allowing the fluorescent molecules to more readily intercalate in these regions than in the G-C regions which have not been disrupted. If this is the case, binding to DNA of fluorescent alkylating agents by attack at the N-7 atom of guanine (as suggested by Caspersson et al. (1969, 1970a)) likely plays little role in producing Q-bands.

Although R-banding still remains very much a mystery, it seems likely that further probing into the distribution of specific non-histone proteins along the length of metaphase chromosomes will yield information very useful in solving the enigma of the mechanism(s) which produce the four types of chromosome banding.

II. "Pre-banding" Cytogenetic Studies of Abortions

Since 1960, some 20 series of "unselected" human spontaneous abortions have been collected and subjected to cytogenetic analysis. This work was recently reviewed by Pawlowitzki (1972) and he reported that the frequency of chromosome anomalies ranged from 3% to 60%. This great variation from one centre to another is to be expected since there are so many selective forces that could or could not be operating in the collection and culturing of any given series.

Firstly, it is virtually impossible to obtain a consecutive series. Early abortions passed at home are lost to research teams depending on hospitals for collection of material. Often specimens received from the hospitals consist only of maternal decidua. On extremely busy days, the hospital staff may not be able to take the few additional steps necessary to save aborted tissue for the research laboratory, or it may be accidently fixed, thereby eliminating the possibility that it will ever grow in culture. Products of conception may be maintained in the uterus so long after death of the embryo or fetus, that the tissue will not grow in culture. The nutrient balance in any culturing medium may allow for growth of tissue with only certain kinds of anomalies.

Researchers also have no control over the fact that the specimens received from any given hospital are subject to the varying selective factors imposed by the socio-economic status of the population served by that hospital. The financial status of a mother could well influence whether or not she will come to a hospital with a very early abortion. Since 80% of abortions occur in the first trimester (Carr, 1972), a decrease in the number of early specimens could easily

alter the conclusions drawn from that study. Mothers under 20 years of age and those over 40 show higher rates of abortion than the intermediate age groups. Both of these groups are proportionately larger in lower income groups. Birth control habits vary according to class and religious belief. Thus an upper-middle class population might produce an increased number of triploid abortions due to conception too soon after oral contraceptive medication, while an inner city hospital might have a high incidence of chromosomally normal abortuses because induced terminations are being cleverly disguised as spontaneous abortions.

Lastly, there is such a thing as too much cooperation from the obstetrical team collecting the abortions. If they are aware of the factors that increase the chances of cytogenetic abnormality, they may submit only those specimens believed to be of interest. Therefore features such as malformation of the embryo, blighted ova (intact empty sacs), swollen villi, increased maternal age and habitual abortion may be unintentionally used to bias the frequency of abnormalities in a so-called unselected series.

It is thus very unlikely that any one study alone has determined the true incidence of chromosome abnormalities in spontaneous abortions. A pioneer in the field, Carr is of the opinion that one-third of all spontaneous abortions are cytogenetically anomalous (Carr, 1972).

Categorizing the aberrations found in 20 studies (all listed in Carr's 1971 paper) Carr found that 42.1% of the anomalies were trisomies, 23.0% were 45,X, 15.5% were triploid, 4.2% were tetraploid and that 14.4% fell into other categories. The distribution of

trisomies, which was determined to be 5.5% in Group A, 3% in Group B, 12% in Group C, 21.5% in Group D, 36% in Group E (on the basis of chromosome morphology, 24% of trisomies were identified as 16-trisomy), 4% in Group F and 18% in Group G, demonstrated that the members of certain chromosome groups (e.g. Group D) were more likely to be found in the trisomic condition than chromosomes belonging to other groups (e.g. Group A).

Many chromosome abnormalities compatible with life have been found to manifest a unique picture clinically. Some examples are trisomy 13 - Patau's syndrome, trisomy 18 - Edward's syndrome, trisomy 21 - mongolism, Down's syndrome, 45,X - Turner's syndrome, 47,XXY - Klinefelter's syndrome, and deletion of the short arm of 5 - cri du chat syndrome.

Polyploid conditions are rarely found in the living human population. The 18 cases of liveborn triploids reported in the literature have shown that low set ears, coloboma of the iris, syndactyly of the hands, a single palmar crease, abnormal male genitalia in XXY individuals, polycystic kidney, congenital heart defect and meningomyelocele are frequently associated with triploidy (Chanbon, 1972; Simpson et al., 1972; Uchida and Lin, 1972; Walker et al., 1973). The most common anomaly, however, is hydatidiform degeneration of a large placenta. There are no documented cases of tetraploidy going to term in humans but tetraploid-diploid mosaicism has been reported three times in live born individuals (Kohn et al., 1967; Atnip and Summitt, 1971; Waller and Waller, 1973).

Attempts to correlate cytogenetics with phenotype have been

less rewarding in abortion studies. This is understandable since in many cases the embryo or fetus is macerated beyond recognition. Curetting may be necessary to remove the products of conception from the uterus in cases of inevitable abortion. Such procedures disrupt intact embryonic tissue. In many cases, it is only a fragment of membrane that is submitted for culturing.

The few generalizations that can be made have been summarized by Carr (1971). In Carr's own study it was found that 40% of all trisomic abortuses were intact, empty sacs, while Roux reported that 50% of his D trisomies had a gross facial anomaly. Phillipe and Boué presented evidence that trisomies C, D, E and G were associated with particular anomalies of the chorionic villi and fetal vessels, although not without overlapping features. Singh and Carr found that only 10% of 45,X specimens were intact empty sacs while 1/3 appeared to be normal and another 1/3 had horse-shoe kidneys. The 45,X ovaries, appearing normal in the embryo, had a decreased number of germ cells in the second half of prenatal life. Lymphangiomas in the neck region were also commonly found in association with this chromosome anomaly. In Carr's series, 85% of triploid specimens presented with hydatidiform degeneration of the chorionic villi. Development was always found to be extremely retarded in tetraploid conceptuses. The embryos were never more than tiny nodules of disorganized tissues.

Before banding patterns were discovered, the definition of syndromes in both the liveborn and abortuses was hampered because many of the chromosomes could not be distinguished from all others in the complement on the basis of morphology or autoradiography (e.g. Groups C, F, G and sometimes E). Since a particular aneuploid

state of one chromosome in a group would produce a different set of characteristics than the same condition of any of the other members of that group, a great deal of variability was found in these "group-syndromes". This is well demonstrated in a review of the cases of C-trisomy mosaicism in the living by Bijlsma et al., (1972).

III. Abortions Studied with Banding Techniques

Since it is now possible to identify each individual chromosome, it seems reasonable to expect that a clinical syndrome will eventually be described for each trisomic condition compatible with livebirth. Such a picutre may already be shaping up for trisomy-8 in the liveborn. Caspersson et al. (1972) have identified 2 cases of pure trisomy-8 in the living. They have also reported 2 cases of trisomy 8 mosaicism as have Bijlsma et al. (1972). Five out of six individuals were mentally retarded. Concomitant strabismus, clinodactyly and skeletal defects each presented 4 times. Three times the skeletal defects included spinal dysraphism. Two had abnormalities of the origin of the heart, while another manifested persistent ductus arteriosus. The mean maternal age was 32.3 years; the mean paternal age - 32.6 years.

Fluorescent banding studies have identified the chromosome abnormality in cat-eye syndrome as partial trisomy 22. The small, supernumerary chromosome in the complement is believed to be responsible for the coloboma, anal atresia, kidney malformation, extreme downward slant of the palpebral fissures, preauricular fistulas and congenital heart disease frequently found in these patients (Bühler et al., 1972).

Reports of chromosome abnormalities in spontaneous abortions, as determined by banding techniques, are now beginning to appear in the literature. A cell strain derived from an intact sac with a slightly macerated 6 mm. embryo was found to be 47,XY,+7 by Giemsa banding (Kuliev et al., 1973).

In three other studies, the aid of Giemsa banding was also enlisted to define the abnormal karyotype. The abortus of a woman who had had 3 miscarriages and no normal pregnancies was identified as 46,XX,-13, +t(13q 13q) (Parslow et al., 1973). Tissue from an intact amniotic sac containing a 15 mm. cord, ending in an amorphous embryonic mass 3 mm. in size, and a yellowish, round, atretic sac 7 mm. in diameter was 47,XX,+14 (Kajii et al., 1972). A 31 x 30 mm. ruptured sac, from which neither cord nor embryo was recovered, was karyotyped as 45,XX,-21 (Ohama and Kajii, 1972).

A series of abortions that occurred during the first 16 weeks of pregnancy, has been analyzed by Lauritsen et al. (1972) using the fluorescent banding technique. Out of a total of 100 specimens cultured, 68 were successfully karyotyped. Thirty-four (50%) of these karyotypes were normal, 15 being 46,XY, while 19 were 46,XX. The embryo data concerning the remaining 34 specimens was not published but the cytogenetic findings were as represented in Table I, page 21.

It is unfortunate that the staining technique of these workers produced some unclear banding patterns which prevented the exact identification of some of the D-, E- and G-group anomalies in this series.

CHROMOSOME ANOMALIES DESCRIBED BY

LAURITSEN ET AL. (1972)

TABLE I

Category of Abnormality	Description within Category	No. of Specimens	Category Total	Category % Total
45,X	45,X	12	12	35%
Trisomy	47,XX,+15	2		
	47,XY,+15	1		
	47,XX,+16	1		
	47,XY,+16	2		
	47,XY,+18	1		
	47,XY,+E_{17-18}	1		
	47,XY,+E	1		
	47,XY,+21	1		
	47,XY,+G	1	11	32%
Triploidy	69,XXX	2		
	69,XXY	1	3	9%
Tetraploidy	92,XXXX	3		
	92,XXYY	1	4	12%
Miscellaneous*	46,XX,-D,+t(13q14q)	1		
	47,XY,+2,Dp+,Gp-	1		
	47,XXY	1		
	48,Xinv (Y),+16,+18	1	4	12%

* The error in nomenclature appearing in the original article has been corrected.

IV. Use of Polymorphic Bands

The detection of banding polymorphisms has pushed the research on spontaneous abortions beyond the simple identification of chromosome abnormalities and syndrome characterization to developmental studies of the origin of these cytogenetic errors.

Polymorphisms can serve as markers to distinguish the maternal chromosome from the paternal chromosome of an homologous pair. If an abortus has a marker which appears in only one of the parents, then it is immediately known whether that chromosome came from the mother or the father. Thus, for certain distributions of markers, it is possible to determine which parent donated the extra set of chromosomes to a triploid abortus.

Markers can also be used to supply information about the mechanism by which the triploid state was created. Non-reduction at the first division of meiosis in the female is indicated when the mother is heterozygous for markers which the father does not have and both of these maternal markers are found in the triploid. Retention of the second polar body is suggested if one of these markers is represented twice in the conceptus. However, if the two markers prove to be paternal in origin, it is difficult to distinguish between fertilization by a diploid sperm and dispermy because not every chromosome has polymorphisms.

Jonasson et al. (1972) have studied 3 triploid abortuses. In one case the origin of the triploidy was determined to be non-reduction during the first division of meiosis in the mother. In the other two cases, the donor of the extra genome could not be identified.

An analysis of the marker chromosomes in a premature triploid infant documented by Uchida and Lin (1972) indicated that the extra set of chromosomes was of paternal origin. In each of three marker triplet sets, the two unique polymorphic forms were found to be identical to those in the father and similar forms were not present in the mother. This is some indication that fertilization was accomplished by a diploid gamete carrying both paternal genomes.

It has been demonstrated that heteromorphic fluorescence patterns can also be used to pinpoint the place and time of occurrence of an error resulting in a trisomic state. In two studies of standard trisomy 21 in the living (Licznerski and Lindsten, 1972; Robinson, 1973) it was possible to determine the origin of the chromosome responsible for mongolism in 5 out of 18 patients. In all 5 individuals, non-disjunction had occurred in the maternal germ cell at the first division of meiosis. Since then, one case of trisomy 21 resulting from maternal non-disjunction during the second meiotic division has been identified (Hutton, 1973). All of the authors concerned have been careful to point out that their conclusions assumed no crossing over in the short arms of chromosome 21.

Banding polymorphisms can also be used to determine the mechanism of twinning in both abortions and liveborns. To date, no such studies have been reported in the literature.

In the research being presented in this thesis, the remains of 27 embryos from 26 spontaneous abortions - one case was a multiple pregnancy - were examined morphologically and cytogenetically. These specimens were carefully selected for the purpose of testing the strength of Q-banding as a technique for obtaining information about the cytogenetics and abnormal development of human spontaneous abortions.

MATERIALS AND METHODS

The spontaneous abortuses described in this thesis were received from St. Joseph's Hospital and Henderson General Hospital in Hamilton, Ontario, between April, 1972, and March, 1973.

The products of conception were either naturally passed or, in cases of inevitable abortion, removed from the uterus by curettage. This tissue was collected in sterile, screw-capped bottles containing physiological saline and was transferred from the hospitals to the university laboratory every weekday.

Only those specimens having characteristics which suggested an increased probability of chromosomal abnormality were selected for culturing. Specifically, these indicators were swollen chorionic villi, an empty sac, less than 12 weeks gestation, gross anatomical abnormalities, habitual abortion and maternal age over 35. If a specimen was to be cultured, this was done the same day as it was received from the hospital.

The tissue cultured was always embryonic membrane. Whenever possible the amnion was separated from the chorion and only the chorion was put into culture. To prevent contamination with maternal cells, the parts of the chorionic villi that might have been in contact with maternal tissue were removed.

When thus isolated from the rest of the specimen, the pieces of chorion to be cultured were placed in 5 ml. of Hanks Balanced Salt Solution (GIBCO) containing 0.5 ml. of antibiotics to give a final

concentration of penicillin G sodium 1,000 I.U./ml., streptomycin 500 µg./ml. and chloramphenicol 50 µg./ml. The tissue was left in this "sterilizing" solution for at least 1/2 hour and then washed in straight Hanks solution. Next it was transferred to a small quantity of fresh Hanks solution, in which it was minced with fine surgical scissors.

All cultures were set up in 3001 disposable tissue culture dishes (Falcon Plastics, Division of Becton, Dickinson, and Co.) and required 2 ml. of culture medium made up according to the following recipe:

100 ml. F-10 Nutrient Medium (GIBCO)

20 ml. Fetal Calf Serum (GIBCO)

1 ml. Penicillin-Streptomycin (GIBCO)

1 ml. L-Glutamine (GIBCO)

The cultures were kept in a CO_2 incubator where the atmosphere was 5% CO_2 and the temperature was maintained at 37° C.

The culturing procedure fell into 3 distinct stages, each with a unique type of culture.

(1) NS Cultures were established when 1 - 3 drops of the minced tissue suspension were put in each of 9 culture dishes and 2 ml. of medium was added. These cultures were left undisturbed in the incubator from 6 to 9 days. During this period, by a process of "natural selection", the viable tissue adhered to the bottom of the dish and gave rise to fibroblasts, while tissue incapable of further growth remained floating in the medium. After the initial "undisturbed" part of the culturing procedure, the NS dishes became subject to the routine of being checked twice a week, as were the other types of cultures described below.

(2) SUB Cultures were set up from NS cultures by the process of subculturing. When sufficient numbers of fibroblasts had grown, fresh medium was put on the NS cultures and the fibroblasts were removed from the bottom of the culture dish by using a rubber policeman. The suspension of fibroblasts was transferred with a siliconized Pasteur pipette to a coverglass, held against the bottom of a culture dish by a drop of Hanks solution. The fibroblasts attached to the coverglass and, after a period of growth, were prepared for chromosomal analysis on this same coverglass.

(3) 1^{o} Cultures grew from the bits of chorion carefully left behind in the original NS dish. When fresh medium was added, this tissue would again settle down and produce more fibroblasts. These fibroblasts could be used to establish more SUB cultures should the first ones fail.

Any slight deviations from this culturing procedure will be noted in the case reports of the abortuses, presented under RESULTS.

When there were enough mitotic figures on one coverglass to allow for cytogenetic analysis of the abortus, the SUB culture was harvested. This involved giving the cells fresh medium 16 - 24 hours before treatment with colcemid (Ciba), which was prepared in the following manner.

One drop of stock solution (0.1% colcemid) was mixed with 4 ml. of Hanks solution and warmed over a spirit lamp to 37^{o}C. Four drops of this solution were put on each culture. After the colcemid was dispersed in the medium by a gentle swirling action, the culture dish was returned to the incubator.

At the end of 5 hours, the medium was poured off the culture and a Pasteur pipetteful of a hypotonic solution of 0.56%KCl at 37°C was carefully put over the cells. The cultures were returned to the CO_2 incubator for 35 minutes after which, each coverglass was transferred to a 1029 disposable Petri dish (Falcon Plastics, Division of Becton, Dickinson and Co.) of fresh 3:1 (absolute methanol: acetic acid) fixative for at least 45 minutes. The coverglass was later removed to a second change of 3:1 fixative and stored under refrigerated conditions overnight or until time was available to finish the preparation.

In the final stages, the coverglass was dipped in fresh, chilled 3:1 fixative and placed on a glass Petri dish. A hair-drier was positioned directly over the coverglass and then the hot air was turned on for 10 - 15 seconds. The coverglass was stored in a disposable 1009 Petri dish (Falcon Plastics, Division of Becton, Dickinson and Co.) until it was required for staining.

The staining procedure was as follows:

Step 1 - 1 dip in fresh distilled water, pH adjusted.

Step 2 - 15 minutes in 0.5% Atebrin (G.T. Gurr), pH adjusted.

Step 3 - 10 minutes in 3 washes of fresh, distilled water, pH adjusted.

Depending on the condition of the preparation, the pH varied from 4.0 to 4.5. Brighter bands were obtained at the higher pH values of this range, but often a lower pH had to be used to decrease the morphological distortion of the chromosomes known as melting.

The stained coverglass was mounted in distilled water at the same pH as the stain and sealed in place with wax. Fluorescence microscopy was conducted using a coverted Zeiss Photomicroscope. Light from the HBO 200 W/4 mercury burner passed through exciter filters BG 38 and BG 12 and barrier filter 47 on a 47 25 47 barrier filter insert. Each slide was scanned with a Planapochromat, 40X, 1.0 N.A., oil, with iris objective. Suitable cells were photographed through a Planapochromat, 100X, 1.25 N.A., oil, with iris objective. These photographs, taken on Kodak high contrast copy film (ASA 64) by a vertical camera attachment, required a mean exposure time of 2 1/2 minutes. Prints were made on F-2 Kodabromide paper. At least 2 cells were karyotyped for each specimen.

After a slide had been scanned, the wax was removed from around the coverglass first, by a razor blade, and then, by a dip in toluene. The slide was frozen so that the coverglass could be removed without disturbing the cells. The coverglass was dipped in absolute ethanol and dried in preparation for permanent staining with carbol fuchsin (Carr and Walker, 1961). After this, it was mounted in Depex.

In order to study the origin of markers found in polyploid specimens, leucocyte cultures of parental blood were set up in the following fashion. 10 ml. of blood was taken in a syringe heparinized with 0.2 ml. of a heparin solution, made by dissolving 70 mg. of sodium heparin (Connaught) in 10 ml. of distilled water. After standing for 5 minutes, the blood was centrifuged for another 5 minutes at 300 rpm. This process yielded about 2 ml. of plasma which was used to set up 2 leucocyte cultures. Both culture bottles contained 0.1 ml. of phytohemagglutinin (Wellcome). To each was added, 8 ml. of MB 597 tissue

culture medium (Connaught), 2 ml. of human AB serum (Hamilton Red Cross) and 1/2 of the plasma. The bottles were stoppered and incubated at 37°C for 72 hours.

At the end of this time, 1 drop of stock colcemid (0.1%) was put on each culture for 3 hours. Then the contents of the culture bottle was transferred by a siliconized pipette to centrifuge tubes and spun for 7 minutes at 600 rpm. The supernatant was pipetted off and a hypotonic solution of 0.56% KCl was gently added. The cells, in hypotonic, were incubated at 37°C for 7 minutes and then spun for 5 minutes at 600 rpm. The supernatant was pipetted off and the button of cells in the bottom of each centrifuge tube was gently broken. After 3:1 fixative was carefully put on the cells, the centrifuge tubes and their contents were refrigerated. Any time after ½ hour of refrigeration, the cells were spun down, resuspended in a small amount of fresh 3:1 fixative and air-dried slides were prepared. Staining and photography were carried out as described for tissue culture chromosome preparations.

RESULTS

I. Histories and Cytogenetic Identifications of Specimens

(i) Normals (Specimens 1 - 11)

Specimen 1 (File No. 88)

An incomplete sac, that appeard to have been dead for some time, was passed around the ninth week of gestation by a 27 year-old woman, reported to be a habitual aborter.

According to fluorescent banding patterns, this specimen had a normal female chromosome complement, 46,XX. Some tetraploid cells were observed in the preparations.

The complete karyotype of this abortion displayed in Figure 1, page 32, demonstrates the typical Q-banding pattern for all normal human chromosomes except Y. This chromosome may be found in the triploid karyotype, Figure 5, page 47.

Specimen 2 (File No. 107)

On examination, the only embryonic material found in this specimen jar was a ruptured sac with a macerated umbilical cord.

Studies of the Q-bands provided conclusive evidence that this abortus had a normal female karyotype, 46,XX.

Specimen 3 (File No. 131)

This specimen included an incomplete sac plus the trunk and upper limbs of an embryo.

The culturing procedure for this sac had to be altered because

Fig. 1. Complete Q-banded karyotype of Specimen 1. Normal female, 46,XX. Direct chromosome preparations of tissue culture fibroblasts, stained with a 0.5% Atebrin solution.

5 specimens with material suitable for culturing were received on one day. Since it seemed after examination that this abortus was more likely than the others to be normal, the tissue selected from it for culturing was refrigerated in 5 ml. of Hanks solution and 0.5 ml. of antibiotics. However, it was noted later that a specimen consisting only of scrapings had come from the same patient 5 months earlier. As a result of the possibility that this mother was a habitual aborter, the tissue that had been stored for 2 days was put into culture.

Only 1 out of 9 cultures grew. It was possible to obtain 3 preparations with adequate numbers of cells by subculturing the fibroblasts from the 1^{o} culture and by saving the cells growing on the bottom of the original subculture dish. The latter was accomplished by transferring the coverglass to be harvested to another tissue culture dish and giving fresh medium to the cells left behind. These fibroblasts then divided several times and were later subcultured.

Only 1 of these preparations provided spreads suitable for analysis. From this preparation the specimen was identified as 46,XX.

Since translocation is one cause of habitual abortion, a careful search for slight alterations in banding patterns was conducted. Due to melted or bent chromosomes in many countable spreads, it was not possible to determine in all cells that all chromosomes had normal banding patterns. However, pooled information on the chromosomal morphology and bands in 6 cells provided no evidence that any of the chromosomes were translocation products.

Specimen 4 (File No. 165)

The pregnancy of a 17 year-old mother produced a full-blown hydatidiform mole.

Tissue from small, fresh-looking vesicles was selected for culturing. It was three weeks before there were enough fibroblasts for setting up subcultures. These subcultures produced very necrotic cells and only 2 out of 9 merited preparation for chromosome analysis.

Not only was the mitotic index very low, but the chromosomes remained clumped together, so that accurate counts could not be obtained. Except for three tetraploid cells, all found close together on the slide, the chromosome number appeared to be diploid. No Y chromosome was observed in any of the spreads. For these reasons, this mole was determined to be 46,XX.

Specimen 5 (File No. 82)

In this case, the products of conception received from the hospital were a ruptured sac with vesicular villi, an umbilical cord, a yolk sac and a macerated embryo measuring 7 mm. in length.

This specimen was cultured in Leighton tubes (Carr, 1971) and in tissue culture dishes as already described in MATERIALS AND METHODS. There was no growth in the Leighton tubes. The tissue put into tissue culture dishes produced an abundance of healthy fibroblasts. An attempt was made to put some of these cells into suspension and prepare air-dried slides. This approach failed. The remaining fibroblasts were successfully subcultured and spreads of good quality were readily found. Some tetraploid cells were observed.

Identification of the chromosomes according to fluorescent banding patterns showed clearly that the abortus had a normal male karyotype, 46,XY.

Specimens 6 and 7 (File Nos. 87-I and 87-R)

This abortus consisted of two sacs. The first was intact and measured 6 mm. in diameter. It contained an amorphous, cream-coloured mass which may have been a tiny, macerated embryo. The second sac, much larger than the first, was ruptured and contained both a fragmented embryo and an umbilical cord.

After staining, it was apparent that both specimens 6 and 7 had the banding patterns of a normal male, 46,XY.

Specimen 8 (File No. 84)

A macerated umbilical cord was attached to a ruptured sac but the embryo was not found.

No explanation can be offered for the extremely poor chromosome preparations produced by cultures of this specimen. A low mitotic index combined with a high incidence of broken cells and poor spreads resulted in some confusion before the abortus was identified as 46,XY.

The assignment of the normal male karyotype was based on two cells in which all of the chromosomes were distinct. Further support was obtained from three other cells where the crucial chromosomes were easily identifiable.

The only complete spread contained one 6 with the typical banding pattern. After all of the other chromosomes but one had been numbered, this one proved to be the right size and shape for a 6 but the banding pattern did not resemble anything in the normal karyotype. Since four other cells displayed two typical 6's, it was concluded that the abnormal pattern did not represent a real difference between the 6's in the cell under discussion. It has been noted in the study of other

specimens that the centromeric region on the long arm of the 6 can be brighter than expected. In this particular case, the opposite has been noted; that is, in one out of five cells the centromeric region has fluoresced much less than expected.

The second good spread was missing a Y. Since the Y appeared in all of the other cells examined, this spread was not considered to represent the complete chromosome complement.

Specimen 9 (File No. 92)

The termination of this pregnancy produced a ruptured sac with an attached macerated umbilical cord. A large mass of clotted blood was also present.

Fibroblasts, grown from this tissue and prepared for chromosomal analysis by fluorescent staining, had a normal male karyotype, 46,XY.

Specimen 10 (File No. 102)

This abortion produced an intact sac enclosed completely by decidua. There was no cord or embryo in the sac. The occurence of an intervillus hemorrhage was noted.

Four of the subcultures became contaminated but the remaining six provided excellent chromosome preparations.

The fluorescent banding patterns corresponded exactly with those of the normal male, 46,XY.

Specimen 11 (File No. 91)

This specimen consisted of a ruptured sac with a macerated umbilical cord. No embryo was found.

A study of the fluorescent banding patterns of the chromosomes of the cultured fibroblasts produced evidence that the genetic constitution

of the abortus was that of a normal male, 46,XY.

(ii) Trisomies (Specimens 12 - 20)

Specimen 12 (File No. 86)

This pregnancy was terminated at an early stage of development by the expulsion from the uterus of a small intact chorionic sac. The amnion and chorion were easily separated, except at the point where the amnion was joined to the chorion by a single stalk. The amnion contained three fluid-filled sacs. There was no trace of an embryo. This blighted ovum appears in Figure 2, page 38.

Two types of cultures were set up for this specimen. The first method has already been described in MATERIALS AND METHODS. In the second procedure, 4 bits of tissue were sandwiched between 2 coverglasses. All of the dishes set up according to the first method became contaminated, but the tissue cultured in the second manner yielded excellent preparations for fluorescent staining.

An extra A group chromosome as represented in Figure 3, Row 1, page 40, was observed in all spreads photographed and using the Q-banding patterns, it was identified as a 2.

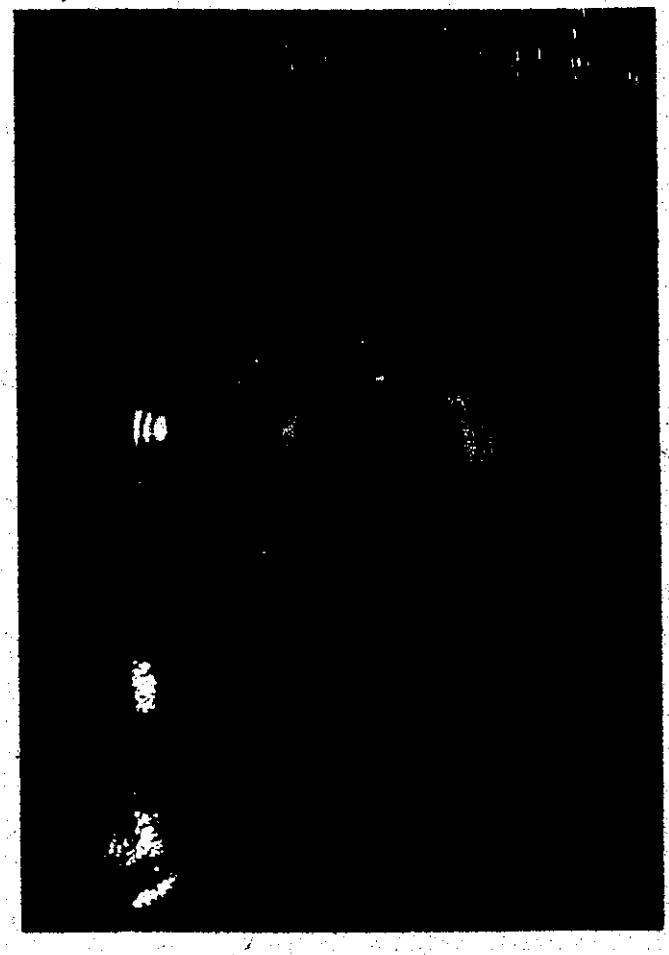
The entire complement of this abortus was described as 47,XX,+2.

Specimen 13 (File No. 93)

The spontaneous termination of a pregnancy in a 43 year old mother provided a ruptured sac with slightly swollen villi. There was no cord or embryonic tissue with the sac.

The fibroblasts that grew from this membrane were necrotic. There were also many round epithelial-like cells in the cultures. 8 out of 9 subcultures became contaminated. However, the remaining culture provided an adequate preparation for chromosome identification.

Fig. 2 Specimen 12, blighted ovum. The amniotic cavity is filled with 3 atretic sacs. No identifiable embryo had formed.


110

The extra C group chromosome shown in Figure 3, Row 2, page 40, was identified as a chromosome 8 on the basis of fluorescent banding patterns. The chromosomal constitution of this abortus was 47,XX,+8.

Specimen 14 (File No. 179)

The macerated tissue associated with this incomplete sac may have been the remains of a very small embryo.

The subcultures grew too slowly to be harvested after 3 days, but by the time of the next routine check most of the cover-glasses had so many cells on them that there was no space left to allow for spreading of the chromosomes. When good spreads were found in these preparations, the chromosomes were often melted, resulting in banding patterns that were not clear.

After much searching for suitable cells, the bands on the chromosomes in these spreads showed that the embryonic material from this abortion was 47,XX,+8. Three 8's are easily identified in the C group of this specimen found in Figure 3, Row 3, page 40.

Specimen 15 (File No. 89)

This very macerated, intact sac, with few villi left on the surface, was found to contain an equally macerated 20 mm. embryo. It was most likely normal, but the limb development could have been retarded. The umbilical cord had false knots. At the time of the abortion, the mother was 42 years of age.

Cell counts indicated that this abortus was trisomic.

Fluorescent staining demonstrated in Figure 3, Row 4, page 40,

Fig. 3 Q-banded partial karyotypes of A, C and D group trisomies.

Row 1: Specimen 12, A group, trisomy 2.

Row 2: Specimen 13, C group, trisomy 8.

Row 3: Specimen 14, C group, trisomy 8.

Row 4: Specimen 15, D group, trisomy 14.

identified the extra chromosome as a 14. The entire complement was 47,XX,+14.

Specimen 16 (File No. 96)

After the spontaneous termination of a pregnancy in a 43 year old woman, a small piece of very old membrane, with attached villi, was available for culturing.

There was only enough tissue for 6 NS dishes. Due to contamination and poor growth, only 4 coverglasses were prepared for cytogenetic analysis.

Counts of 47 chromosomes were noted in many cells. In 4 cells, three chromosomes with the typical 16 banding pattern were found. Because chromosome quality and spreading were poor, it was not possible to obtain a complete E group for karyotyping.

As 2 X chromsomes were identified in most cells and since no Y chromosome was seen in any of the spreads, the chromosone complement of this abortus was determined to be 47,XX,+16.

Specimen 17 (File No. 94)

The wastage from this pregnancy was identified as a ruptured sac with swollen and club-shaped villi. No cord or embryonic material was found. When she aborted, the mother was 42 years old.

The culturing of this tissue was successful, with the exception that one plate became contaminated.

The preparations of this specimen displayed excellent spreads. Melting was difficult to control even at pH 4.0. Nevertheless photographs were obtained of 6 excellent cells. All of these cells displayed three small metacentric E group chromosomes with the banding

pattern of a 16. In Figure 4, Row 1, page 44, one of the 16's appears larger than the other two 16's. This is likely explained by the fact that it was on the periphery of the spread, thus having more room to spread than the chromosomes in the centre.

This abortus was cytogenetically defined as 47,XY,+16 on the basis of Q-bands.

Specimen 18 (File No. 100)

At the time that this pregnancy terminated, the mother had been taking oral contraceptives for five months. The products of conception consisted of a ruptured sac. No remnants of cord or embryo were identified.

The cultures set up for this specimen demonstrated clearly that it is not good to be overly generous with the amount of tissue used in the NS dishes. In this case, the medium had turned very yellow during the settling down and early growth period. The fibroblasts established under these conditions were necrotic and the mitotic index for each culture was low. After the medium was changed and the dead, floating tissue thus removed, the cells became more healthy. Some plates had so much growth that it was possible to obtain two subcultures from one NS dish. All of those subcultures grew very well, the only exception being one dish which was discarded due to contamination.

These preparations provided good spreads in which the extra chromosome seen in each cell was readily identified as a 16. The chromosomes pictured in Figure 4, Row 2, page 44, were photographed

from a preparation stained at pH 4.0. Although the fluorescence was somewhat pale, the difficulty of karyotypic analysis was not increased by the problem of distorted chromosome morphology due to melting.

Using fluorescent banding patterns, this abortus was classified as 47,XY,+16.

Specimen 19 (File No. 182)

This specimen consisted of an incomplete sac. No tissue was identified as being part of either the cord or the embryo.

All plates were ready for subculture the first time they were checked. Within three days, the coverglasses to which the fibroblasts had been transferred were completely covered with healthy cells displaying a high mitotic index.

The rapid growth of this specimen resulted in so many cells on each coverglass that it was difficult to get good spreads. Even in spreads where all of the chromosomes were distinct, they were often bent so that the banding patterns were obscured. To make the identification of this specimen more certain, the same cells that had been studied by fluorescence were photographed again after permanent staining with carbol fuchsin.

In Figure 4, Row 3 (top), page 44, one of the 16's is bent so that the banding pattern is ambiguous. Carbol fuchsin staining demonstrates that this chromosome has the shape characteristic of a 16.

Only one of the E group chromosomes in Figure 4, Row 3 (top), page 44, displays the three bands peculiar to chromosome 17. After

Fig. 4 Q-banded partial karyotypes of E and G group trisomies.

Row 1: Specimen 17, E group, trisomy 16.

Row 2: Specimen 18, E group, trisomy 16.

Row 3(top): Specimen 19, E group, trisomy 16. Q-banded.

Row 3(bottom): Specimen 19, E group, trisomy 16. Stained with carbol fuchsin.

Row 4: Specimen 20, G group, trisomy 22.

staining with carbol fuchsin, the chromosome showing only the bright band at the end of the long arm appears identical in shape and size to the chromosome with the typical 17 banding pattern.

It was clearly demonstrated in three cells studied by both staining procedures that the chromosomal constitution of this abortus was 47,XY,+16, with one 17 displaying an atypical fluorescent banding pattern.

This case is a good example of a situation in which the information from fluorescent banding alone is not sufficient to allow for the pairing of all of the chromosomes in the complement.

Specimen 20 (File No. 130)

About three-quarters of this intact sac was covered with decidua. The rest of the surface was bare. There was no cord or embryo found inside the sac.

It was a month from the time that the specimen was put into culture until the first dish was ready to harvest. The fibroblasts of the subcultures yielded excellent spreads of chromosomes that could easily be studied.

From figure 4, Row 4, page 44, it is evident that the extra G group chromosome bands as a typical 22.

On the basis of the Q-banding pattern alone, the abortus was identified as 47,XX,+22.

(iii) Polyploids (Specimens 21-27)

Specimen 21 (File No. 98)

Cultures of this abortus were established using tissue from an incomplete sac. It was noted that some of the villi attached to the sac were vesicular. The obstetrical history of the mother indicated that this spontaneous abortion marked the termination of the second pregnancy of a 22 year old woman. Her first pregnancy, occurring approximately 5 years before had produced an abnormal, 1380 gram, 2 weeks post-dates female infant, who died a few hours after birth. Multiple congenital anomalies included tracheo-esophageal fistula, horseshoe kidney, bony deformities and cardiac abnormalities.

As a result of poor growth and some contamination, only 4 TS dishes were subcultured. These cultures, however, provided sufficient material to allow for the cytogenetic identification of this specimen.

The fluorescent karyotype of the entire chromosome complement, as shown in Figure 5, page 47, clearly demonstrates the triploid nature of this specimen, which was defined as 69,XXY.

Specimen 22 (File No. 97)

Examination of the contents of the specimen bottle revealed a ruptured sac with vesicular villi measuring approximately 3 mm. X 2 mm.

All cell counts were in the triploid range. The sex chromosomes are pictured in Figure 6, Row 1, page 49.

With the aid of fluorescent banding patterns, this abortus was identified as 69,XXY.

Fig. 5 Complete Q-banded karyotype of Specimen 21. Triploid, 69,XXY.

Specimen 23 (File No. 104)

An intact chorionic sac, measuring 1 cm. in diameter, was found embedded in a massive hemorrhage. An embryo, which looked normal, occupied most of the cavity. However, normal development could not be confirmed, since the degree of maceration of the tissues was so great that the embryo fell apart on being touched with forceps.

Counts indicated a triploid number of chromsomes in the cultured fibroblasts.

Figure 6, Row 2, page 49, represents the sex chromosome constitution of all of the cells studied. The entire complement of this specimen was thus determined to be 69,XXY.

Specimen 24 (File No. 129)

It was easily visible to the naked eye that the villi attached to the chorionic sac were swollen. Abnormal development in the 17 mm. embryo, shown in Figure 7, page 50, had resulted in ectopia cordis and herniation of the liver into the umbilical cord.

All spreads counted were in the triploid range.

The Q-bands of the sex chromosomes, as demonstrated in Figure 6, Row 3, page 49, indicate that the abortus was 69,XXY.

Specimen 25 (File No. 85)

This specimen was a full-blown hydatidiform mole, the largest vesicle being 2 1/2 cm. X 1 1/2 cm. The tissue was very macerated and appeard to have been dead for a long time.

Fig. 6 Q-banded Sex Chromosomes of Polyploid Specimens.

Row 1: Specimen 22, 69,XXY.

Row 2: Specimen 23, 69,XXY.

Row 3: Specimen 24, 69,XXY.

Row 4: Specimen 26, 92,XXXX.

Row 5: Specimen 27, 92,XXYY.

Fig. 7 Specimen 24, abnormal 17 mm. embryo. Ectopia cordis and herniation of liver into umbilical cord. 69,XXY.

tentative de s'épanouir en tant qu'individu et de retrouver les sources authentiques de son peuple. A travers le roman Agaguk, le personnage principal s'oppose toujours à son père Ramook, qui se révèle comme un homme ambitieux, malhonnête et corrompu. Il en va presque de même dans le roman Tayaout. Le fils Tayaout, parti vers un nouvel horizon, contraste avec le père Agaguk qui, enraciné dans le village, se corrompt. Tayaout devient plus imbu des rites et des traditions esquimaux que son père. Il cherche la solitude et la liberté des pays glacés. Son éloignement du village rappelle celui de son père et ainsi rappelle le cycle de la vie de celui-là. Lui autrefois était jeune et indépendant; il s'est éloigné de la tribu pour voyager et s'établir ailleurs. Mais plus tard il est revenu au village où il s'est enraciné et a renié sa vie d'autrefois. Maintenant c'est son fils qui reprend le fil. A son tour il part, pour revenir plus tard à la vie du groupe.

Mais poussons plus loin cet examen du cycle chez Thériault. Dans Agaguk et surtout dans Tayaout l'éloignement des héros et leurs voyages vers le Nord remettent le temps cyclique en mouvement. Les personnages thérausiens témoignent tous de ce même désir de renouvellement dans leurs voyages au dos de la Terre. Plus jeune et sans doute pas encore ambitieux ni corrompu, Ramook a amené son fils à la pêche aux Sommets du Monde où vivent les derniers Esquimaux. Agaguk imite ce geste paternel en faisant ce même voyage avec son fils et sa femme. Et à son tour le fils (et petit-fils)

Tayaout, suivant le même geste ancestral, part à la conquête des Sommets.

Quant aux rêves et aux projets personnels d'Oonak et de Tugugak — deux notables de la tribu — de s'en aller vers le Nord, ils traduisent aussi leurs efforts de recommencement. Tous semblent avoir un grand respect pour les Esquimaux qui y vivent pendant toute l'année.

Cette notion du cycle, du recommencement selon un modèle archétypal dans les romans esquimaux de Thériault, coïncide étroitement avec les conclusions de l'étude de Mircea Eliade sur les sociétés traditionnelles dans son livre Le Mythe de l'éternel retour. Dans son avant-propos il nous apprend que:

> Un trait nous a frappé en étudiant ces sociétés traditionnelles: c'est leur révolte contre le temps concret, historique, leur nostalgie d'un retour périodique au temps mythique des origines, au Grand Temps.[1]

Dans leur éloignement de la vie du village nous notons chez Agaguk et chez Tayaout la volonté de rejeter le temps concret pour retrouver leurs racines dans le passé. Et comme le constate Eliade,[2] cette opposition n'est pas simplement l'effet des tendances conservatrices des sociétés primitives: c'est plutôt un véritable désir de retourner au temps des origines:

> Pour l'homme primitif tout acte est posé et vécu antérieurement par un autre...ce qu'il fait a déjà été

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[1] Mircea Eliade, Le Mythe de l'éternel retour (Paris: Editions Gallimard, 1969), p. 9. Nous soulignons.

[2] Loc. cit.

> fait. Sa vie est la répétition ininterrompue de gestes inaugurés par d'autres.[1]

Dans cette optique nous comprenons mieux la conception de Tayaout comme réceptacle des idées et des traditions infuses par toutes les générations. Nous voyons plus clairement dans son imitation des archétypes comment ce jeune Inuk, imbu de nostalgie, semble être "vécu", comment son recommencement est "semblable à tous les recommencements".[2] La notion du temps surtout au début de Tayaout semble être abolie:

> "J'habite le Sommet du Monde. J'y suis depuis des millénaires l'homme continuel, je suis sans âge parce que j'ai tous les âges. Je suis sans traces de l'ancêtre parce que je suis l'ancêtre en même temps que la continuation..."[3]

Selon Eliade on remarque dans les sociétés traditionnelles l'abolition du temps historique par l'imitation des archétypes et par la répétition des gestes paradigmatiques.

> L'abolition du temps profane et la projection de l'homme dans le temps mythique ne se produisent qu'aux intervalles essentiels, c'est-à-dire...au moment des rituels ou des actes importants.[4]

D'autre part l'idée de cycle réapparaît dans le choix même du nom, "Tayaout". Choisir un tel patronyme symbolisant espoir et ambition signifie un certain désir de renouvellement des gestes et des actes de ce noble ancêtre et reflète l'impératif intérieur du père de retrouver ses origines.

---

[1] Ibid., p. 15.

[2] Thériault, Tayaout, p. 11.

[3] Loc. cit.

[4] Mircea Eliade, op. cit., pp. 49-50.

of the cells were in the tetraploid range. There were remarkably few overlaps in the spreads considering the large number of chromosomes in each cell.

Figure 6, Row 4, page 49, provides the evidence that led to the classification of this specimen as 92,XXXX.

Specimen 27 (File No. 95)

The only product of conception available for study in this case was a fragment of chorionic sac with attached villi. It was noted on the hospital requisition form that, at the time of the abortion, the mother was taking oral contraceptives.

The tissue selected for culture grew very slowly. The fibroblasts thus established appeared necrotic. One out of nine NS dishes was discarded because it became contaminated. The subcultures of the remaining dishes grew, but contamination resulted in the termination of all but one culture.

From this one preparation it was possible to determine that the number of chromosomes in each cell was in the tetraploid range. No diploid cells were observed. It was not possible to get perfect spreads of all 92 chromosomes from this preparation and the quality of the fluorescent bands on the chromosomes was only mediocre.

Figure 6, Row 5, page 49, displays the sex chromosomes of two cells cultured from the chorionic sac of this abortus. The entire complement was described as 92,XXYY.

CYTOGENETIC CONSTITUTION OF EMBRYONIC REMAINS

FROM 26 HUMAN SPONTANEOUS ABORTIONS

TABLE II

	# of Specimens	% Representation in Series
NORMALS		
46,XX	4	11/27 = 41%
46,XY	7	
TRISOMICS		
47,XX,+2	1	
47,XX+8	2	
47,XX,+14	1	9/27 = 33%
47,XX,+16	1	
47,XY,+16	3	
47,XX,+22	1	
POLYPLOIDS		
69,XXY	4	
69, ? (male)	1	7/27 = 26%
92,XXXX	1	
92,XXYY	1	

PHENOTYPES OF 27 ABORTED EMBRYOS

TABLE III

Type of Chromosome Abnormality	NORMAL EMBRYO OR FETUS		ABNORMAL EMBRYO OR FETUS		BLIGHTED OVUM*		HYDATIDIFORM MOLE		INCOMPLETE SPECIMEN	
	Chromosomes		Chromosomes		Chromosomes		Chromosomes		Chromosomes	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
--	1				2		1		7	
Triploidy		1 (?)				2				6
Triploidy		1 (?)		1				1		2
Tetraploidy						1				1

* Blighted Ovum - intact sac, either empty or containing tissue that indicates severe retardation of embryo development.

II. Developmental Studies

(i) Using Markers in Twins (Specimens 6 and 7)

As shown in Figure 9, page 57, the variable regions in all three of the D group chromosomes seem to be identical in the fibroblasts cultured from both sacs. The very bright satellites on one of the 15's provide an extremely powerful marker, especially since the other 15 has pale short arms and no satellites. Neither of the 14's are satellited, but one member of the pair has a medium bright band at the end of the short arm. This does not appear in the homologue. In the 13's, the pale satellites are separated from the short arm by a larger gap in one chromosome than the other. This indicates a difference in length between the non-fluorescing stalks of the two 13 chromosomes.

(ii) Using Markers in Triploids

1) Specimen 21

The chromosomes prepared from leucocyte cultures of parental blood indicated that the parents of Specimen 21 were cytogenetically normal. This family study was difficult. There was no striking marker in either parent, such as the very bright satellites found on one of the 15's of Specimens 6 and 7. Also, the cell-to-cell variation in the intensity of the short arm bands on the D group chromosomes proved to be confusing. Under these circumstances the following criteria had to be used to determine the origin of the extra set of chromosomes.

In 5 out of 5 paternal cells, both 13's had a band on the short arm which fluoresced as 2 bright spots. Pale stalks could be seen on one of these chromosomes (13-P1) in each cell, but not on the other 13 chromosome (13-P2). See Figure 10, Row 1, page 58.

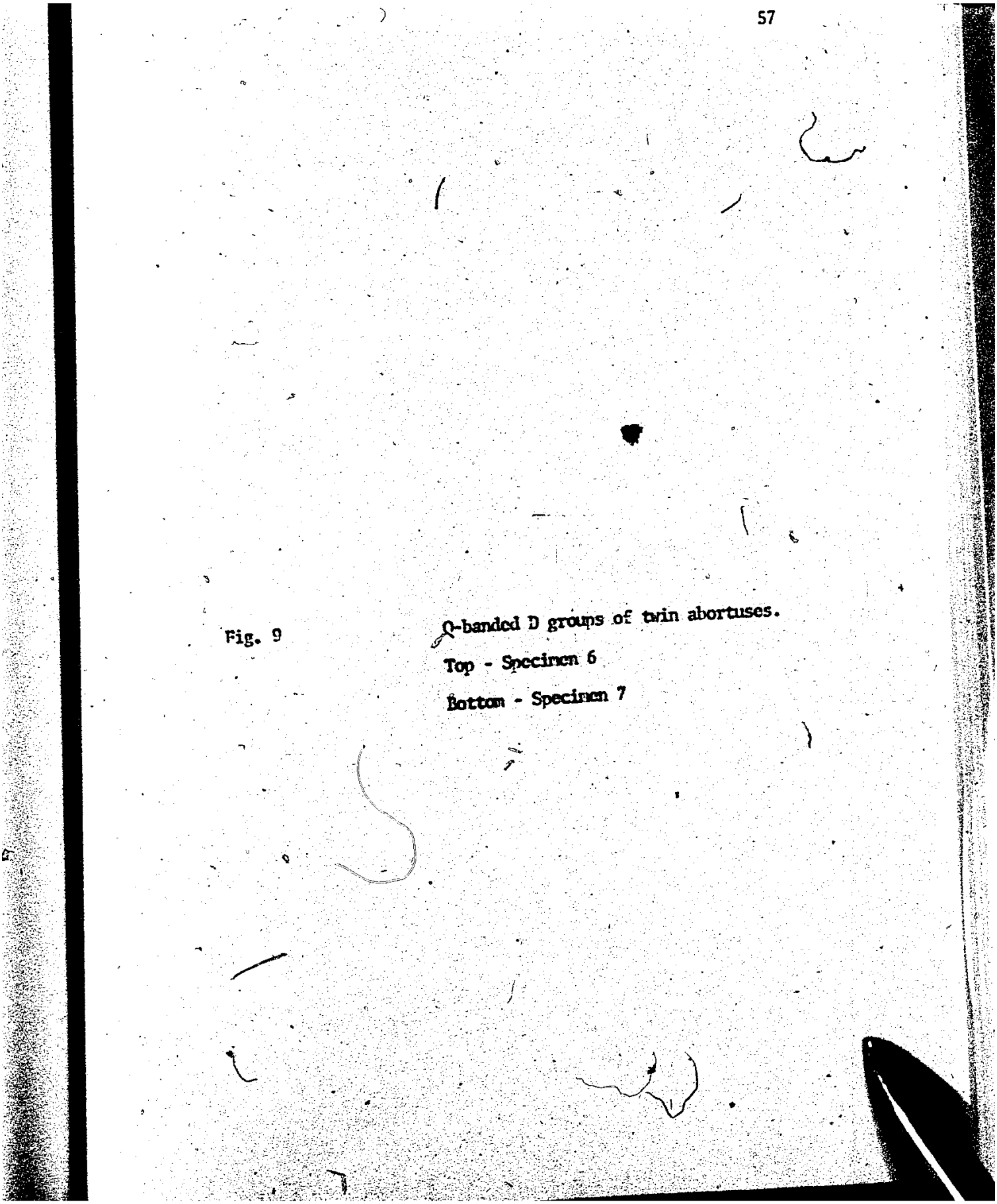


Fig. 9 Q-banded D groups of twin abortuses.

Top - Specimen 6

Bottom - Specimen 7

Fig. 10 Triploid family studies.

Diagrammatic representation of marker chromosomes.

Row 1: 13's of mother (left) and father (right) of Specimen 21.

Row 2: 13's of Specimen 21.

Row 3: 13's and 14's of mother and "father?" of Specimen 22.

Row 4: 13's and 14's of Specimen 22.

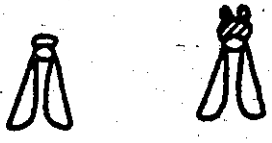
Key

bright fluorescence

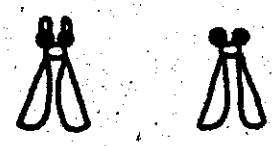
medium fluorescence

pale fluorescence

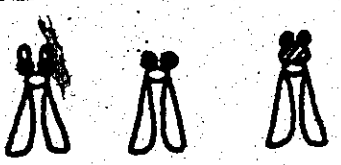
N.B. Fluorescence patterns are indicated for short arms only.



13-M1 13-M2

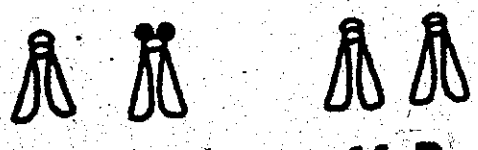


13-P1 13-P2



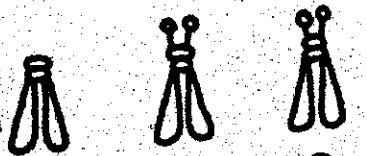
13-P1 13-P2 13-M2

13-M1 13-M2 13-P1 13-P2



14-M1 14-M2 14-P

13-M2 ? ?
X-1 X-2



14-M1 or 14-P ? X-3 ? X-3

Eight out of nine maternal cells displayed one 13(13-M1) with no distinguishing band in the variable region. The one chromosome, in which there did seem to be a short arm band of notable intensity, was long and the increased brightness may well have been due to the bending of the short arm back on itself.

The other maternal 13(13-M2) had a band on the short arm less bright than those found on 13-P1 or 13-P2. Immediately above this band were stalks only slightly less intense in fluorescence than the band itself. This band appeared as 2 bright spots in only 2 out of 9 cells. Thus, the maternal 13's were determined to be as diagrammed in Figure 10, Row 1, page 58.

In 8 out of 9 triploid cells, there were two bright spots on the short arms of 2 of the 13's. The pale stalks of chromosome 13-P1 showed up above these bright spots in one of the 13's in 5 cells. Chromosome 13-M1 did not appear in any of the triploid cells, while careful scrutiny showed 13-M2 to be present in 9 out of 9 cells karyotyped. The 13's of this abortus are represented in Figure 10, Row 2, page 58.

2) Specimen 22

The chromosomes of the parents of Specimen 22, prepared from leucocyte cultures, were also found to be normal. A close examination of the variable regions of the six 13 and 14 chromosomes found in the abortus, revealed three chromosomes that could not be found in either of the parents. In the written description immediately following and in Figure 10, Row 4, page 58, these "phantom" chromosomes have been referred to as X-1, X-2 and X-3.

In one paternal chromosome 13 (13-P1) the bright band in the short arm always appeared as two round bodies. Its homologue (13-P2) had brightly fluorescing satellites and a short arm with a band of medium intensity.

The father's 14's had identical banding patterns. Both members of the pair 14-P had no band on the short arm and no stalks or satellites. The father's 13's and 14's are shown in Figure 10, Row 3, page 58.

The maternal 13's are described as follows: 13-M1 had a bright band on the short arm and bright satellites, separated from the rest of the chromosome by short stalks. 13-M2 had two bright dots on the short arm, longer stalks than 13-M1 and pale satellites.

The maternal chromosome labelled 14-M1 was identical to 14-P, described above, while 14-M2 had two bright dots on the short arm and no stalks or satellites. See maternal 13's and 14's diagrammed in Figure 10, Row 3, page 58.

The triplet of 13's in the abortus displayed one chromosome identical to 13-M2. Of the remaining two chromosomes, neither had satellites, but the first had a bright band on the short arm in 7 out of 7 cells, while the second, identified in 6 out of 8 triploid cells, had no such band. These chromosomes were designated X-1 and X-2 respectively. X-1 was considered to be different from 13-P1, in which the bright area on the short arms appeared as 2 dots in all cells observed.

One of the triploid's 14's was identical to 14-M1 and 14-P. Exact identification of the other two 14's was difficult. Chromosome X-3 had pale satellites and a short arm with no band. In 4 out of 8 cells

studied, 2 of the 14's were X-3's, while in 6 out of 8 cells, at least 1 of the 14's had the pattern of this third chromosome which was not detected in either of the parents.

DISCUSSION

I. General: Application of Banding to Abortion Studies

The modification of the fluorochrome banding technique, which allowed it to be used regularly for human chromosome analysis, greatly expanded the outlook of cytogeneticists studying chromosome anomalies in human spontaneous abortions.

It had been shown that trisomies 2, 3 and 16, positively identified because of the distinguishing morphological features of these chromosomes, occurred in abortions but never in livebirths, while chromosomes 13, 18 and 21, in the trisomic state, were consistent with survival after birth but were rarely found in abortuses. Would certain of the chromosomes that could not be identified by unique arm length and centromere position now be found associated with spontaneous abortion more frequently than others?

Cohen and Putnam (1972) reported that a retarded girl with multiple congenital anomalies, who had been identified as 45,XX,G- using the conventional Giemsa stain, was actually 45,XX,+t (18p21q) when her chromosomes were subjected to Q- and G-banding techniques. The error arose because, in the initial karyotype, arranged according to morphological features, the translocation chromosome had appeared within the limits of variation allowed to normal E group members and thus, one G chromosome was thought to be completely missing from the complement. Such a demonstration of the increased accuracy in chromosome

identification afforded by banding patterns raised questions concerning the rarity of translocations found in spontaneous abortions. Would reciprocal translocations previously masked, because no morphological difference could be detected between the translocation products and normal chromosomes, now become apparent?

Apart from the actual identification of anomalous chromsomes, it was hoped that much could be learned about abnormal development through the use of Q-banding polymorphisms on homologous chromosomes. What information would these markers provide in studies of twinning? Would non-disjunction producing trisomies be more common in female than in male germ cells? To what stage in meiosis would non-reduction of chromosome number most often be traced in studies of the origin of polyploidy? Could it be determined which parent would be most likely to donate the extra genome(s)? Would it be possible to find polymorphisms in each chromosome pair, so that there would be a means of distinguishing between dispermy and fertilization by a diploid sperm, when the extra set of chromosomes was of paternal origin?

While the present study of 27 aborted embryos does not touch on all of these questions, it nevertheless demonstrates the usefulness of the Q-banding technique in the identification of the chromosome complements of spontaneous abortions and provides new cases to be added to the pool of some 75 specimens, that have already been analyzed by the various banding techniques. It supports the commencement of full-scale investigations into the origin of polyploidy, but, at the same time, has brought into the open the obstacles that will be encountered in such studies.

II. Chromosome Anomalies and Accompanying Abortus Phenotypes

(i) Trisomies

The present study presents the first 2 cases of trisomy 8 to be identified in spontaneous abortions. Since 2 living patients with pure trisomy 8 have also been documented (Caspersson et al., 1972), it appears that chromosome 8 is not one of the chromosomes, which in the trisomic state, creates such an imbalance within the cell, that the embryo dies long before term and is always spontaneously aborted.

Until now, positive identification of trisomy 22 in aborted material had not been made. This condition is not known in the living, but the small, supernumerary, satellited, submetacentric chromosome associated with the cat-eye syndrome has been identified by fluorescence as a deleted 22 (22q-). Since individuals with one normal 22 and one 22q- appear phenotypically normal (Bühler et al., 1972), there must be very little critical genetic information at the end of the long arm of chromosome 22. From this, it follows that, genetically, the aborted trisomy 22 and the living partial trisomy 22 are very similar. Thus trisomy 22 conceptuses may abort or produce viable individuals.

The trisomy 14 found in this series was the second abortus to be so identified. Trisomy 14 has not been identified in the living, so it may well be that the balance of genes on a pair of 14's is so critical to the functioning of a cell, that one extra 14 invariably results in the death of the embryo early in pregnancy.

As in previous studies, chromosome 16 was the one most often responsible for the trisomic condition (4 times out of 9).

CHARACTERISTICS OF TRISOMIES IDENTIFIED IN THIS STUDY

TABLE IV

Specimen #	Genetic Constitution	Specimen Complete (C) or Incomplete (I)	Further Specimen Description
12	47,XX,+2	C	blighted ovum
13	47,XX,+8	I	vesicular villi, maternal age 43
14	47,XX,+8	I	assumed to be under 12 weeks gestation
15	47,XX,+14	C	maternal age 42, macerated embryo appearing normal; limb development may have been retarded
16	47,XX,+16	I	maternal age 43
17	47,XY,+16	I	vesicular villi, maternal age 42
18	47,XY,+16	I	mother taking oral contraceptive medication at time of conception
19	47,XY,+16	I	assumed to be under 12 weeks gestation
20	47,XX,+22	C	blighted ovum

The trisomies identified in this series and the characteristics responsible for their initial inclusion in the study are listed in Table IV, page 65. This table shows that the characteristic most frequently associated with the trisomic specimens was increased maternal age. In this series, any specimen from a woman over 35 years of age was cultured, regardless of morphological features. (It should be noted that all of the women in the "over 35" category in this series were also over 40). Only 4 such specimens were selected, but on cytogenetic analysis, all of them were shown to be trisomic. This supports the theory of predisposition of aged ova to non-disjunction.

To date chromosomes 2, 3, 7, 8, 14, 15, 16, 18, 21 and 22 have been positively identified in the trisomic state in spontaneous abortions. As more and more abortuses are karyotyped by banding patterns, this list is likely to grow. However, it is remarkable that although chromosome 1 can be distinguished from all of the other chromosomes on the basis of morphology alone, not one case of trisomy 1 has been found among some 1500 specimens that have been cytogenetically analyzed. Could it be that this product of conception always dies before implantation, so that the mother of a trisomy 1 is never aware that a pregnancy has occurred? Or does chromosome 1 carry a gene which controls its segregation so well that non-disjunction is virtually impossible? If this is the case, it may be that chromosomes 19 and 20, which are rarely found in the trisomic condition, possess segregation-control genes that have evolved to a state only slightly less effective than that on chromosome 1, while the counterpart on chromosome 16 is in a relatively primitive form, since three copies of this chromosome appear frequently in spontaneously aborted tissue.

(ii) Triploids

Of the five specimens proven to be triploid, three were distinguished by hydatidiform degeneration, as determined by the swollen condition of the chorionic villi, and one was a full-blown hydatidiform mole. Neither of these peculiar placental pathologies were noted in the remaining triploid, Specimen 23. The embryo in Specimen 23 seemed normal at first glance, but further examination was impossible because the tissue was so macerated that it fell apart on being touched. Thus, as with other chromosome anomalies, the concomitant morphological picture varies, but should an abortus-triploid syndrome be found to exist, one of the major characteristics will be swollen chorionic villi.

The abnormal embryo found in triploid Specimen 24 presented with ectopia cordis. This pathology is the result of a fusion defect and it may be significant that other fusion defects such as meningomyelocele, cleft lip and palate and coloboma of the iris have been frequently found in triploid infants surviving to term. Mittwoch and Delhanty (1972) found that in a diploid/triploid mixed fibroblast culture, established from a girl karyotyped as a 46,XX/69,XXX mosaic, the triploid cells had a slower mitotic rate than the diploid cells and that the triploid cells gradually disappeared from the cell population over a period of five weeks. This gradual decline of triploid cells could explain fusion defects in pure triploid abortuses or liveborns. If the only difference between triploid and diploid cells were the increased time required to complete the cell cycle in triploid cells, then the triploid embryo should form normally, but be smaller than the diploid embryo. However, if each hour, even a few more triploid cells die than would be the case with normal diploid cells, then there might well not be enough healthy triploid cells left to

accomplish closure during critical periods of development and fusion defects would result.

(iii) Tetraploids

In accordance with earlier works, the only identifiable tetraploid embryo in this series was a tiny nodule of disorganized tissue, grossly retarded in development. Tetraploidy has never been found in a liveborn human, except in association with a line of cells known to be compatible with livebirth. This indicates one basic difference between the plant and animal kingdoms. Plants seem to have evolved a mechanism for putting extra genomes through the cell cycle without influencing the rate of division or life expectancy of the cell prerequisite for normal development. In fact, polyploidy in plants is often responsible for increased vigour. In man and other animals, it may be that tetraploid cells have the same problems as triploid cells, but in an exaggerated form. That is, tetraploid cells may have even a longer cell cycle and undergo even fewer divisions before death than triploid cells. As a result, the pure tetraploid conceptus shows little or no resemblance to the normal diploid embryo.

At this point, it is convenient to digress slightly, to discuss a few general aspects of the determination of the tetraploid condition in vitro. Tetraploidy is known to arise spontaneously in diploid cultures (Carr, 1971). Milunsky et al., (1971) have found, as have many other laboratories, that extensive polyploidy (mostly tetraploidy) in amniotic fluid cell cultures appears to be compatible with the birth of a clinically normal child. In their particular case, 9 pregnancies, with 4 - 43% polyploidy, all produced normal children. Thus, in the study here under discussion, a low percentage of tetraploid cells in diploid cultures was not regarded as

diploid/tetraploid mosaicism and abortuses were identified as being pure tetraploids only if no diploid cells were observed in conjunction with the tetraploid strain.

III. Indicators of Chromosome Abnormality in Abortions

The characteristic most frequently found to be the forerunner of the identification of an abnormal chromosome complement was vesicular villi. This condition of hydatidiform degeneration was described in 6 specimens; 3 of these were triploid, 2 were trisomic and one had a normal karyotype. Since it is possible that hydatidiform degeneration is the prelude to hydatidiform mole, but simply aborted before the full-blown mole can develop, it is appropriate to mention here that of two moles cultured, one was triploid, while the other was diploid. Researchers, who found full-blown moles were more likely to be diploid than triploid, hypothesized that diploid specimens were maintained longer in utero so that the full-blown mole had a chance to develop, while those with a triploid genome were expelled in the early stages of chorionic degeneration before the condition was fully manifested (Carr, 1971).

Mention has already been made of the fact that, in this series, all mothers over 40 years of age produced trisomic abortuses.

Out of 5 blighted ova cultured, 2 proved to be trisomic and another one was tetraploid. It is generally considered that 50% of blighted ova have cytogenetic abnormalities.

It has been noted that there is a marked increase in the incidence of triploidy in abortions of women who conceived within 6 months of discontinuing oral contraceptive medication (Carr, 1972). One possible explanation was that the hormone balance in the mother's body had not had sufficient time

to return to normal. When the hospital requisitions, received with two of the specimens collected during the present study, indicated that conception had occurred while the mother was on "the pill", these specimens were cultured because it was definitely known that such abortuses, while in utero, were in an environment hormonally different from that favouring motherhood. Both were found to be abnormal; one being trisomy 16 and the other tetraploid. It is also of interest that both of these conditions are incompatible with livebirth. In depth cytogenetic studies of abortions of women conceiving while on "the pill" have not been conducted. It would be interesting to know if different chromosome anomalies are consistently found depending on the length of time that the mother has been off medication.

Since the incidence of translocation carriers in couples experiencing recurrent abortion is more than 10 times greater than that found in the general population (Carr, 1972), it was hoped that translocations would be detected in the conceptuses passed by women known to be habitual aborters. However, in this series, the abortuses from two habitual aborters both were normal, indicating that the cause of these particular abortions was not a chromosome abnormality.

The most outstanding difference between this study of spontaneous abortions and all others is the total lack of 45,X specimens. This can be explained because in this series no morphologically normal fetus, over 12 weeks gestation and having normal chorionic villi was cultured. It is known that one third of 45,X specimens appear normal and most survive beyond 12 weeks since only 10% are classified as blighted ova. Also hydatidiform degeneration is not characteristic of the 45,X condition. Thus 45,X specimens were definitely selected against and it is reasonable that none were identified in this research.

IV. A New Polymorphism?

Analysis of Specimen 19 (47,XY,+16) showed that one of the 17's had an atypical Q-banding pattern. The Q-positive band at the end of the long arm was present as usual, but those on either side of the centromere did not show up.

Carbol fuchsin staining of cells previously Q-banded was done to assess the morphology of the atypical chromosome. The ends of chromosomes often do not fluoresce, thus making a chromosome seem shorter than it actually is. Staining with carbol fuchsin helps to clarify this situation since all parts of the chromosome - except the regions of secondary constriction - take up this dye equally well.

Figure 4, Row 3, page 44, shows the E group of Specimen 19, taken from a cell stained first with Atebrin and then with carbol fuchsin. The 17 on the left appears a little shorter simply because it is bent at the centromere. It should also be noted that the Q-banded chromosomes were photographed at a lower magnification than those stained permanently. Taking these factors into consideration, both of the 17's seem to have the same morphological features. Since all of the other chromosomes have typical Q-banding patterns, the possibility of a reciprocal translocation is eliminated.

This atypical chromosome was identified in all spreads in which both 17's were found. It is then most unlikely that the occurrence of this 17 is an artifact due to staining variability in the cell photographed in Figure 4, Row 3, page 44.

The cytogenetic analysis of the parents of Specimen 19 required to establish that this 17 is a variant occurring in the normal karyotype has yet to be done. Should this banding pattern be established as a

normal variant, 17 will become the first chromosome to have a heteromorphic banding pattern (not due to re-arrangement) in a region of the chromosome not classified as being heterochromatic.

V. Developmental Studies

(i) Using Markers in Twins (Specimens 6 and 7)

The zygosity of a twinning event cannot always be determined by the number of chorionic sacs. Monozygous twins are generally thought to have a common chorionic sac, but it is possible that a division of the blastomeres very early on in development could produce two completely separate chorionic sacs, even though fertilization involved only one ovum and one sperm. On the other hand, dizygous twins, resulting from the approximately simultaneous fertilization of two different ova by two different sperm, could appear to have a common chorion if the blastocysts implanted so close that the individual chorions fused.

Since monozygous twins should have identical markers, while dizygous twins would not, heteromorphic banding patterns provide a means of discriminating between these two types of twinning events, even in the absence of information about the condition of the chorion(s) which may be misleading because of distortion caused by the abortion or birth process.

In Specimens 6 and 7, each pair of chromosomes in the D group is heterozygous for certain band polymorphisms. The fact that these polymorphisms are identical in tissue derived from two separate chorionic sacs is by no means enough evidence to allow a conclusion about the zygosity of the twins to be drawn. After all, there are 19 other autosomal pairs (both specimens were of the same sex - male) that

possibly are not identical in both embryos. However, it is an indication that it is unlikely that twinning originated from the fertilization of two separate ova by two different sperm. Rather, it seems more reasonable that fertilization was normal and that, at a very early stage of development, the blastocyst divided to give rise to two separate chorionic sacs.

(ii) Using Markers in Triploids

1) Background

The use of markers which appeared after conventional staining techniques were employed was very limited. Ferrier et al. (1964) were able to identify the presence of large satellites and a large secondary constriction on two of the nine D group chromosomes in the triploid cell line of a diploid-triploid mosaic male patient. Since the father showed no satellited D chromosomes, they assumed that the mother had donated the extra genome, although her chromosomes were not studied. But this conclusion could be disputed since it is known that satellites do not stain consistently. Also, they may stain but go unobserved because they are bent under the rest of the chromosome. Both of these problems are found with Q-banding. However, in the cells where the satellites do stain and are not hidden from view, the bands are of the same intensity from cell to cell.

Although five triploid specimens were identified in this series, the parental karyotypes, required for analysis of the origin of the polyploid condition, were available in only two cases.

2) Specimen 21

It seems reasonable to propose that at conception Specimen 21 received chromosomes 13-P1 and 13-P2 from its father and 13-M2 from its mother. See Figure 10, Rows 1 and 2, page 53. From this point, it may

be extrapolated that the rest of the extra set of chromosomes was also of paternal origin.

Since both paternal 13 chromosomes were present, a normal ovum must have been fertilized by a diploid sperm containing both marker 13's or by two sperm, each one carrying a different marker 13. A diploid sperm could arise from non-reduction of chromosome number at the first meiotic division (assuming that a functional sperm can differentiate in spite of the initial error) or by the fusion of two spermatids. In the first case the genome would be the same as that found in the somatic cells of the father. Genetically, the second case would be equivalent to disperny. Until a marker can be identified on every chromosome, it will not be possible to positively distinguish between fertilization by a diploid sperm containing the entire genome of the father and disperny.

3) Specimen 22

The discovery that four out of the six 13 and 14 chromosomes in this triploid abortus could not be traced to either parent was indeed baffling. One of the 13's, 13-M2, is of maternal origin. It is unlikely that X-1 and X-2 are the products of a cross-over between 13-P1 and 13-P2, the father's 13's, since the bright satellites of 13-P2 do not appear in either X-1 or X-2. However, little is yet known about the effect on banding patterns of crossing-over near regions of secondary constriction.

14-P and 14-M1 are identical. This chromosome is also found in the abortus. X-3, present twice in the triploid, displays satellites consistently, while there are never any satellites on the 14's of either parent. Unless satellites can be distinguished more readily in tissue

culture preparations than in air-dried slides of blood cultures, or they are more likely to be identified after crossing-over, the only explanation for these "phantom" markers is that the male in this "family" study was not the father of the abortus. Assuming this to be the case, the traceable 14 must be 14-M1 and the extra set of chromosomes of male origin.

Since the genetic constitution of the real father is not known, the presence of two X-3 chromosomes in the abortus could be due to the father actually being homozygous for X-3. It is equally as probable that X-3 was the only 13 of a heterozygous, homologous pair involved in the abnormal fertilization event. Thus, X-3 could have been present twice in the diploid sperm which accomplished fertilization or have been carried by both sperm involved in dispermy.

VI. Cell-to-Cell Variability in Heteromorphic Bands

Unless striking markers, such as the 15 in Specimens 6 and 7, are present, analysis of variable regions is very time consuming and requires much careful thought. Artifactual variability from one photograph to the next may prove confusing, but if all of one chromosome pair from as many cells as possible are compared, the variability can be understood in terms of the most likely banding pattern and the heterozygous forms of homologues can be characterized.

The quality of the preparation greatly affects the intensity of Q-banding. If two chromatids lie over each other, intensity is greatly increased. Satellites, which could be detected, may be bent under the rest of the chromosome and thus missed on analysis. If bright satellites bend under a Q-negative band, a new Q-positive band will seem to have been formed. If they bend under a Q-positive band, fluorescence of that

band will be increased. The difference between a bright area on the short arms of acrocentrics appearing as 2 separate dots or as a band completely crossing both chromatids may be due to nothing more than a separation of chromatids, causing the transverse band to be broken in some spreads. However, this difference often is consistent from cell to cell within one individual, in which case it is unlikely that the difference is an artifact of the preparation.

SUMMARY

As a result of this research project, Q-banding has been shown to be an accurate and convenient means of identifying individual chromosome anomalies in spontaneous abortions. It is too soon to determine if further abortion syndromes will be identified as a result of this refined technique. Polymorphic bands have proved to be useful in tracing the sequence of events producing abnormal developmental patterns. It is hoped by the present author that the discussion of cell-to-cell variability of these polymorphisms will serve as a warning that such variations must be very carefully analyzed before conclusions about previous development can be drawn. Nevertheless, there is every indication that much important information will be forthcoming as a result of the use of Q-banding in the study of aneuploidy, polyploidy and multiple births in man.

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