HALL, JOAN OVERBY. A Cytogenetic Investigation of Selected Children with Birth Defects. (1969) Directed by: Dr. Laura G. Anderton. pp. 115

Cytogenetic investigation of 46 selected children with birth defects was carried out by culture of peripheral leukocytes to determine (1) whether a chromosomal anomaly could be demonstrated, (2) the correlation between karyotype and phenotype, and (3) dosage effect in cases of aneuploidy by electrophoresis of serum proteins.

This study revealed 20 abnormal chromosome complements and one XY sex chromosome complement in a phenotypic female. There was some individual variation in phenotype among those affected by a given chromosomal anomaly, probably due to differences in genetic background and in maternal host environment. Abnormalities in development common to two or more different chromosomal anomalies were also noted, mental deficiency being common to all. However, there was a relatively consistent and distinct overall pattern of development (phenotype) with each chromosomal abnormality, which should allow a specific clinical diagnosis.

Electrophoretic studies of serum proteins in a female with four X chromosomes, normal XX females, and normal XY males failed to show a dosage effect, indicating that genes for synthesis of serum proteins are not located on the active region(s) of the "inactivated" X chromosome(s).

A CYTOGENETIC INVESTIGATION OF SELECTED CHILDREN WITH BIRTH DEFECTS

by

Joan Overby Hall

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Arts

Greensboro
April, 1969

Approved by

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APPROVAL SHEET

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ACKNOWLEDGMENTS

To Dr. Laura G. Anderton, I am grateful for the provision of research facilities and materials, and for invaluable advice throughout this research and preparation of the thesis.

I am indebted to William W. McLendon, M.D., Associate Pathologist, and to the staff pediatricians at Moses H. Cone Memorial Hospital, and to John D. French, M.D., of the Children's Medical Center, Martinsville, Virginia, for their cooperation in making available the patients for this study.

I am deeply grateful for the patience and understanding of my husband, Roger, and son, Jeff, without which this undertaking would not have been possible.

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INTRODUCTION

This study is a cytogenetic investigation of selected children with birth defects, screened at Moses H. Cone Memorial Hospital, Greensboro, N. C., and at Children's Medical Center, Martinsville, Va. Lenz (1966) has grouped genetic and partially genetic defects into five different categories:

1. Monogenic diseases with identified biochemical defect
2. Monogenic abnormalities of tissue structure
3. Disorders of embryonic development (malformations)
4. Polygenic functional disorders
5. Chromosomal aberrations

The primary purpose of this investigation was to determine whether a chromosomal anomaly could be demonstrated in each of the 46 patients selected by physicians on the basis of phenotypic abnormalities (birth defects).

The second purpose of this study was to correlate phenotype with karyotype (chromosome pattern). Geneticists and embryologists, working with various plants and animals, have been concerned with this task. Penrose (1966) has reviewed this literature and has pointed out that "the action of a whole chromosome, or of a large segment, can be rather less specific than the action of a single gene." It is therefore necessary to determine variations in phenotype for a given karyotype, and to determine phenotypic abnormalities common to different karyotypes. An effort was made to elicit the causes of these variations in

phenotype for a given karyotype.

The third purpose of this study was to investigate dosage effect in cases of aneuploidy (abnormal number of chromosomes) by determining biochemical changes measured by electrophoresis of serum proteins.

This investigation was to be both informational and useful to the physician. If the metabolic defects in each chromosomal anomaly could be determined, perhaps the patients could be treated in order to control the defects. Certain metabolic defects have been associated already with chromosomal anomalies, e.g., persistence of embryonic hemoglobin in trisomy D_1 syndrome (Huehns et al., 1964) and increased leukocyte alkaline phosphatase in trisomy G_1 syndrome (King, Gillis, and Baikie, 1962). With the newly developed techniques of cytogenetic examination and treatment of fetuses in utero, perhaps chromosomal anomalies could be detected early enough and treatment initiated before brain damage and developmental anomalies occur.

MATERIALS AND METHODS

Peripheral Leukocyte Cultures

A modification of the micro procedure of Moorhead and Mellman (1965) was used for peripheral leukocyte cultures in the study of metaphase chromosomes.

The skin was cleaned with 70% alcohol, and blood was obtained using sterile technique. Two to three drops of whole blood were placed into each of three vials containing 5 ml of culture medium as described in the Appendix. The vials were swirled to disperse the blood in the medium, and, after being transported from the hospital or physician's office to the Biology Department of the University of North Carolina, Greensboro, were incubated at 37°C for 4 days (approximately 96 hr.). The cultures were left undisturbed except when adjustment of pH to approximately 7.1, as indicated by phenol red, became necessary. The pH was raised by replacing the plastic cap with a sterile cotton plug for a few minutes to allow the excess CO_2 to escape. The pH was lowered by adding sterile 0.1 N HCl.

After incubation for 4 days, 0.02 cc of a 0.85% saline solution of colchicine, 100 µg/ml (Sigma Chemical Company), was added to each vial to arrest mitosis at metaphase, and the vials were swirled to mix thoroughly. Incubation was continued for approximately 3 hr. to allow metaphases to accumulate.

Harvesting of Leukocyte Cultures

Leukocyte cultures were harvested according to the following

procedure, a modification of that used by Moorhead and Nowell (1964):

1. Swirl vials to suspend cells in medium, then transfer contents to a 15-ml conical centrifuge tube.
2. Centrifuge at 1200 rpm (International Centrifuge, Model HN) for 10 min.
3. Remove supernatant fluid, add 8 to 10 ml of Hanks' BSS (Microbiological Associates, Inc.) at room temperature, and resuspend cells by aspirating with a serum dropper pipet to rinse off the culture medium.
4. Centrifuge as before.
5. Remove all but 0.5 ml of supernatant fluid and resuspend cells.
6. Add 2.0 ml of distilled water, resuspend cells in this volume, and allow cells to stand in this hypotonic solution for 8 to 10 min. to rupture the cell membranes and cause the chromosomes to swell.
7. Resuspend cells and centrifuge as before.
8. Remove all of the supernatant fluid.
9. Add 1 ml of fresh fixative consisting of glacial acetic acid and absolute methanol (one:three), avoiding disruption of the pellet of cells. Allow to stand for 30 min.
10. Suspend cells in the fixative by repeated aspiration.
11. Centrifuge as before.
12. Discard the supernatant fluid and suspend cells in a second portion of fixative.

13. Continue changes of fixative until the supernatant fluid is colorless and a fine suspension of cells is obtained.
14. Remove a new, precleaned slide from iced distilled water, place on a rack at a 45° angle, and drop one drop of the cell suspension at the upper end.
15. Ignite over a flame and allow the blaze to become extinguished to accomplish spreading of chromosomes.
16. Shake the slide vigorously for a few seconds to hasten drying.

Staining of Chromosomes

The dried slides were stained with a Giemsa mixture, according to the following procedure (Moorhead and Nowell, 1964):

1. Hydrolyze in 1 N HCl at 60°C for 10 min.
2. Rinse in distilled water.
3. Stain in fresh Giemsa mixture, consisting of 90 ml distilled water, 10 ml Giemsa stain, and 7 ml 0.15 M NH_4OH for 8 to 12 min.
4. Dehydrate through two changes of acetone, with three to four dips in each change.
5. Following a 2-min. period in acetone-xylene (one:one), clear in two changes of xylene.
6. Mount, using a clear permanent mounting medium and a No. 1 coverglass (Corning).

Examination of Metaphase Chromosomes

A sufficient number of metaphases (24 to 200) was counted to establish the modal number of chromosomes of each patient, using

1000 x oil immersion magnification. Structural abnormalities, such as gaps and breaks, were noted, as well as such phenomena as satellite association and somatic pairing of chromosomes.

A minimum of six metaphases was selected to photograph for preparation of karyotypes. Kodak Plus-X-Pan Professional film was used, with insertion of a wide band interference filter (Carl Zeiss, Inc.) into the optical system. After development of the film according to instructions included with the individual package, enlargements were printed onto Kodak Kodabromide F . 4, 8 X 10-inch photographic paper.

Karyotypes were prepared by cutting out the chromosomes, matching the pairs, and arranging them, according to the Denver system of classification, into seven groups, A to G.

Sex Chromatin Studies

Oral mucosa smears were taken after discarding the first scraping containing dead cells and debris. They were fixed with Spray-cyte (Clay-Adams, Inc.) and stained using the Feulgen technique described by McManus and Mowry (1960), which is specific for DNA. A minimum of 100 cells was examined for the presence of sex chromatin (Barr bodies). The nuclei to be included had to meet the following criteria: flat, oval, unobscured, and well stained, with finely dispersed chromatin and unbroken nuclear membrane; only deeply stained, clearly defined chromatin masses approximately 1 μ or greater in diameter and situated against the nuclear membrane were classified as sex chromatin (Hsu, Klinger, and Weiss, 1967).

Peripheral blood smears were stained with Wright stain, and 100 to 1000 polymorphonuclear neutrophils were examined for sex chromatin

appendages ("drumsticks"). Only deeply stained appendages approximately 1 μ in width and attached to the nucleus by a thin filament were classified as sex chromatin.

Electrophoretic Separation and Quantitative Determination of Serum Proteins

Polyacrylamide gel was used as the supporting medium for electrophoresis. The disc technique of Davis (1964), for vertical separation of minute quantities of proteins, was used for this study. All sera were promptly separated from the clot, frozen immediately, and maintained at -15°C. The gels were prepared according to procedures described by Davis, omitting a sample gel. First the separation gel was prepared, then the spacer gel. Sera were diluted with 40% sucrose so that 250 μg of protein was contained in 0.2 ml; total protein had been determined by SMA-12 at Moses H. Cone Memorial Hospital. The diluted sera (0.2 ml) were applied to the spacer gels and layered with TRIS glycine buffer, pH 8.3. Electrophoresis proceeded for 55 min. at 44 ma (Power Supply by Buchler Instruments, Model 3-1014A). The gels were removed from the tubes, fixed and stained in 1% amido black in 7% acetic acid for 1 hr. Destaining was accomplished by leaching with four to five changes of 7% acetic acid every 4 to 6 hr. except overnight, with continuous agitation on a mechanical shaker at lowest speed. The protein bands were quantified using a recording densitometer (Densicord by Photovolt) with integrator (Integraph Model 49 by Photovolt); a 595 filter and range D-1 were used.

RESULTS

Forty-six patients were selected by the Pathology Department of Moses H. Cone Memorial Hospital and by the Children's Medical Center for chromosome analysis on the basis of clinical (phenotypic) abnormalities. The results of the chromosome analyses are listed in Table 1 below.

Table 1. Results of chromosome analyses

Karyotype*		Number of patients
47,XX,G +	(47 chromosomes, XX sex chromosomes, an additional G group chromosome)	7
47,XY,G +	(47 chromosomes, XY sex chromosomes, an additional G group chromosome)	7
46,XY/47,XY,G +	(a chromosome mosaic with a normal male cell line and a cell line with an extra G group chromosome)	1
47,XX,18 +	(47 chromosomes, XX sex chromosomes, an additional chromosome no. 18)	2
45,X	(45 chromosomes, a single X sex chromosome)	1
47,XXY	(47 chromosomes, XXY sex chromosomes)	1
48,XXXX	(48 chromosomes, XXXX sex chromosomes)	1
46,XY	in a phenotypic female	1
46,XX	(normal female karyotype)	15
46,XY	(normal male karyotype)	10

* Chicago Conference, 1966, designation

Clinical Report 1

1. Case no.: 7 2. Age: 5 weeks 3. Date of birth: 28 May 1966

4. Race: White 5. Sex: female 6. Date blood taken: 7 July 1966

7. Age of mother at birth of propositus: 36

Present state of health: living and well

8. Age of father at birth of propositus: 49

Present state of health: living and well

9. Siblings: two half brothers by mother, ages 12 and 15, living and well; six half siblings by father, living and well

10. Conceptual history: third pregnancy

11. History of pregnancy: large doses of thyroid and weight control medication; no prenatal illness

12. Labor: normal

Delivery: term, low forceps

13. Birth weight: 8 lb. 11½ oz. Length: 20½ inches

Head circumference: 13-3/4 inches Chest: 13½ inches

Respiration: difficult

14. Physical examination and clinical history

Physical examination at birth revealed a moderately obese infant with fairly marked mongoloid slanting of eyes and with epicanthal folds, webbing of neck attributable to obesity and loose skin, low-set ears, short hands with normal creases, thumb that was turned around, and poor grasp. The infant became cyanotic and required oxygen. The physician's impression was probable mongolism with questionable congenital heart disease. A cardiac consultation at the age of 1 week revealed no evidence of congenital heart disease.

During the first year of life there were three hospital admissions. The first, at age 6 months, was because of croup. The second, at age 9 months, was because of acute extensive bronchitis with possible beginning pneumonitis; at this time a heart murmur was noted, as well as a small umbilical hernia. The third admission, 11 days later, was because of bacillary dysentery (Salmonella).

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells				24		24
% occurrence				100%		

Seven karyotypes showed 47 chromosomes with trisomy in group G (Fig. 1, page 12). These findings were consistent with a diagnosis of sporadic mongolism (Down's syndrome).

Figure 1. Karyotype of case no. 7

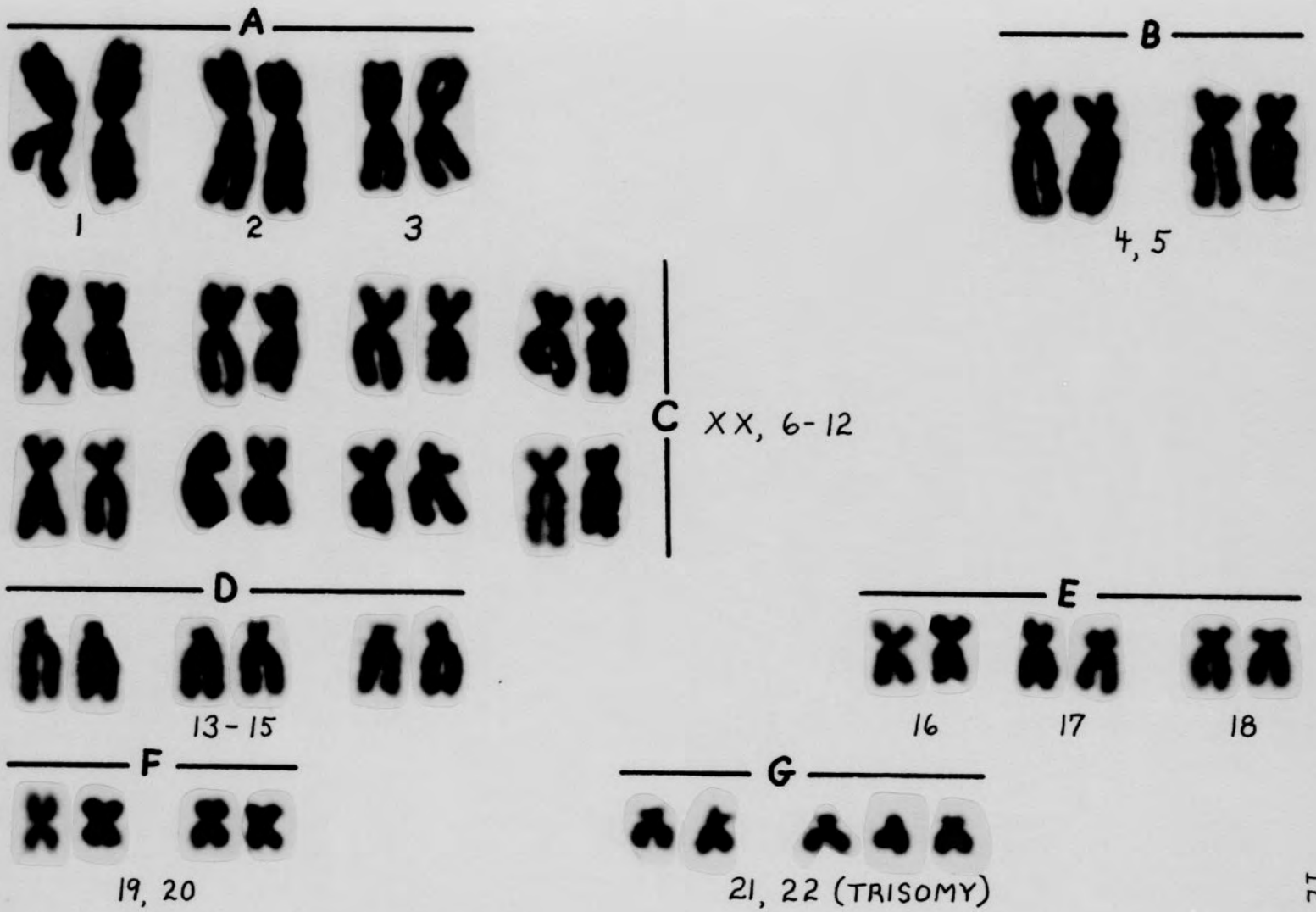
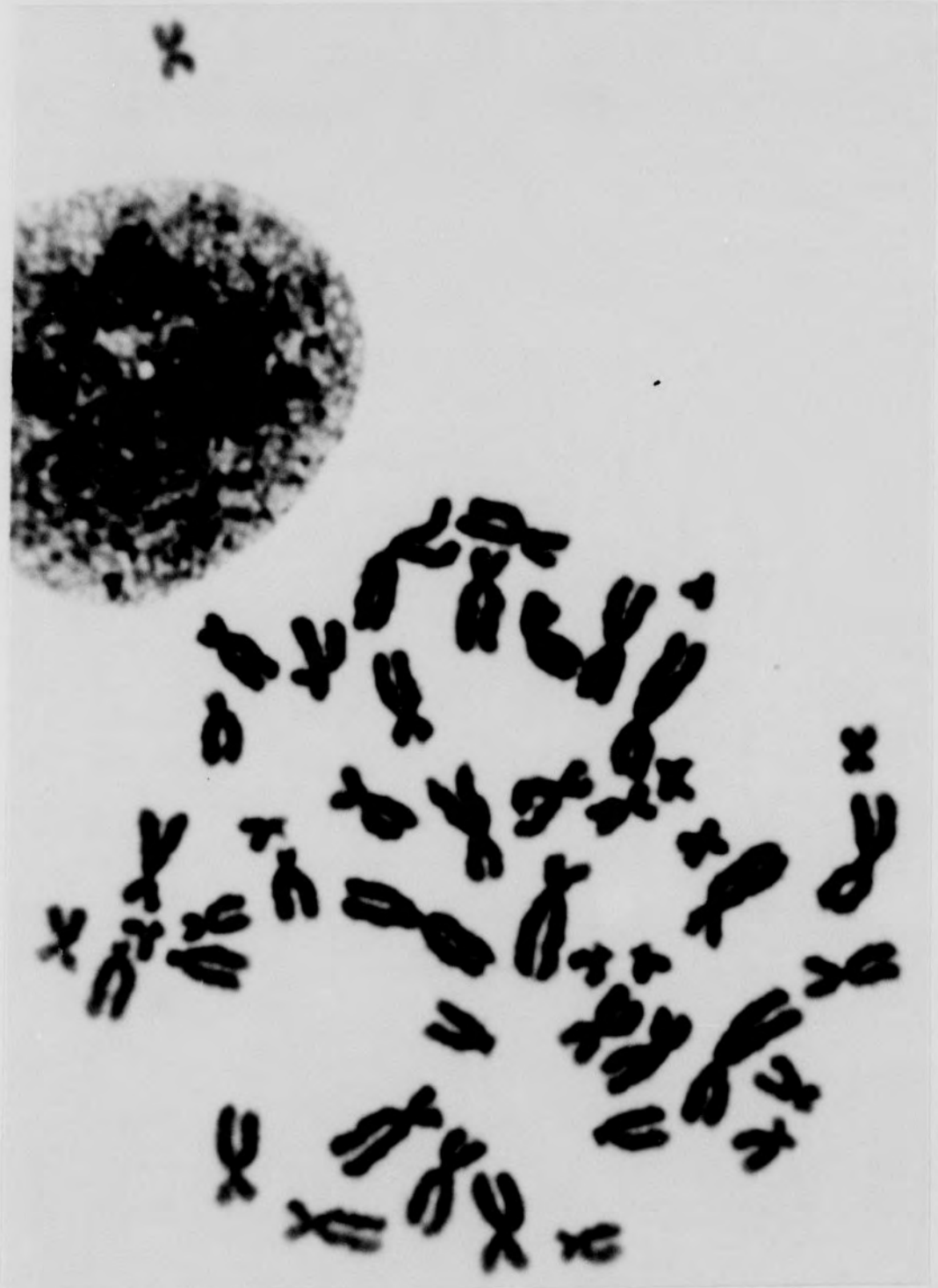

A
B
1
2
3
4, 5
C XX, 6-12
D
E
13-15
16
17
18
F
G
19, 20
21, 22 (TRISOMY)

Figure 2. Metaphase chromosome spread of karyotype, Fig. 1



Clinical Report 2

1. Case no.: 38 2. Age: 2 months 3. Date of birth: 18 May 1968

4. Race: White 5. Sex: male 6. Date blood taken: 19 July 1968

7. Age of mother at birth of propositus: 19

Present state of health: good; family history of congenital heart disease, cleft palate, failure to thrive (three cousins)

8. Age of father at birth of propositus: 28

Present state of health: good

9. Siblings: none

10. Conceptual history: first pregnancy

11. History of pregnancy: uncomplicated

12. Labor: N. R.*

Delivery: premature (7 months gestation)

13. Birth weight: 4 lb. 1 oz. Length: N. R.

Head circumference: N. R. Chest: N. R.

Respiration: N. R.

14. Physical examination and clinical history

Physical examination at birth revealed a somewhat unusual appearance, felt to be familial. During the first check-up, the pediatrician noted unusual facies with some stigmata of mongolism. There were slight epicanthal folds, the ears were small and slightly low-set, and the infant tended to keep the tongue protruded. There were no simian creases. During the second month a heart murmur was first noted.

* Not recorded

The first hospital admission, at age 6 months, was because of acute laryngitis; slight hyperextensibility of the extremities was noted at this time. The second hospital admission, at age 8 months, was also because of acute laryngitis. A cardiac consultation at this time revealed congenital heart disease with a left-to-right shunt at the atrial level.

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells	4	21	100	74	1	200
% occurrence	2.0%	10.5%	50.0%	37.0%	0.5%	

Eight karyotypes with 46 chromosomes showed a normal male chromosome complement (Fig. 3, page 18); eight karyotypes with 47 chromosomes showed trisomy G (Fig. 5, page 22). These findings indicated chromosome mosaicism with a normal cell line and a cell line with trisomy G, as seen in sporadic mongolism.

Figure 3. Karyotype of case no. 38

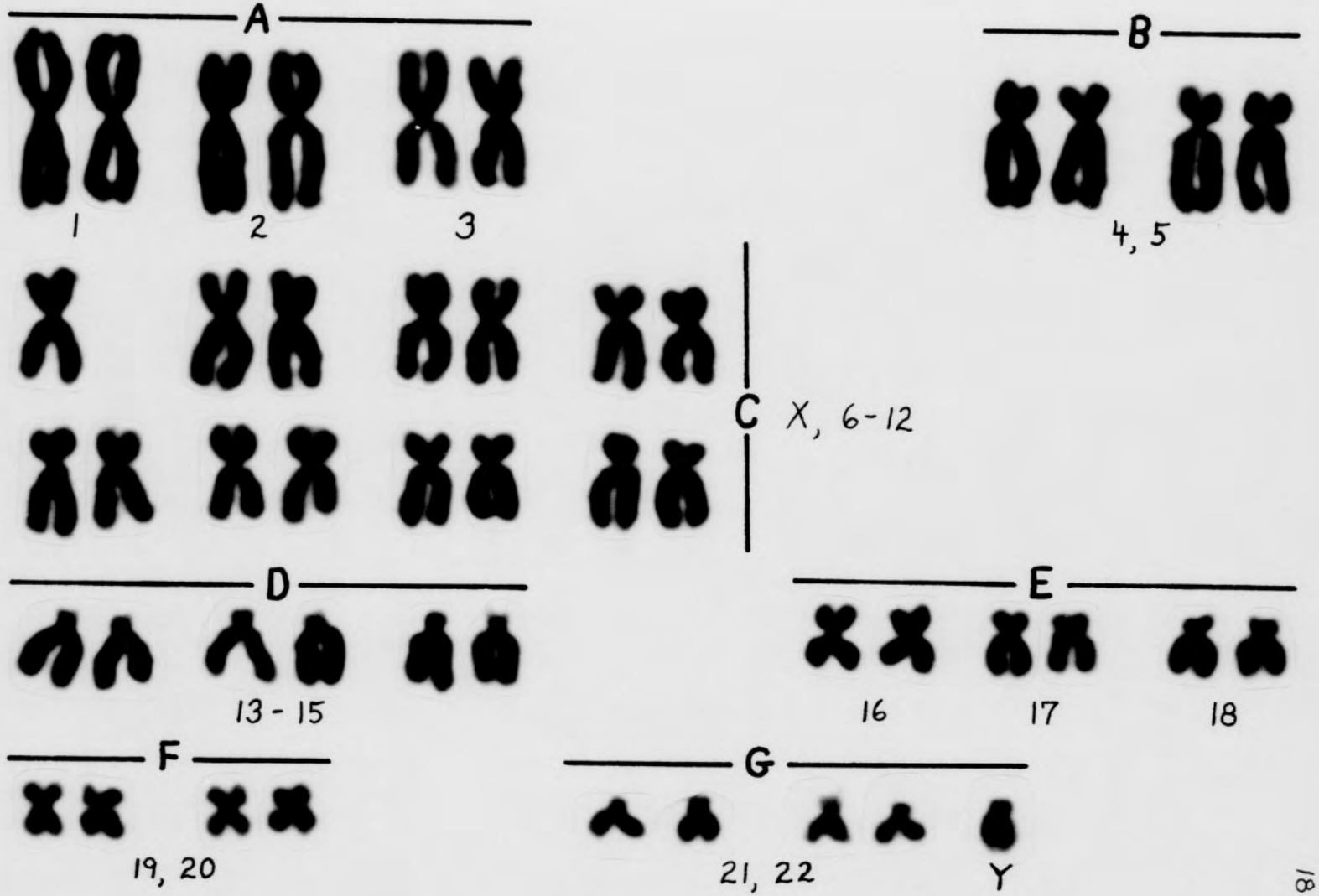

A
B
1
2
3
4, 5
C X, 6-12
D
E
13 - 15
16
17
18
F
G
19, 20
21, 22
Y

Figure 4. Metaphase chromosome spread of karyotype, Fig. 3

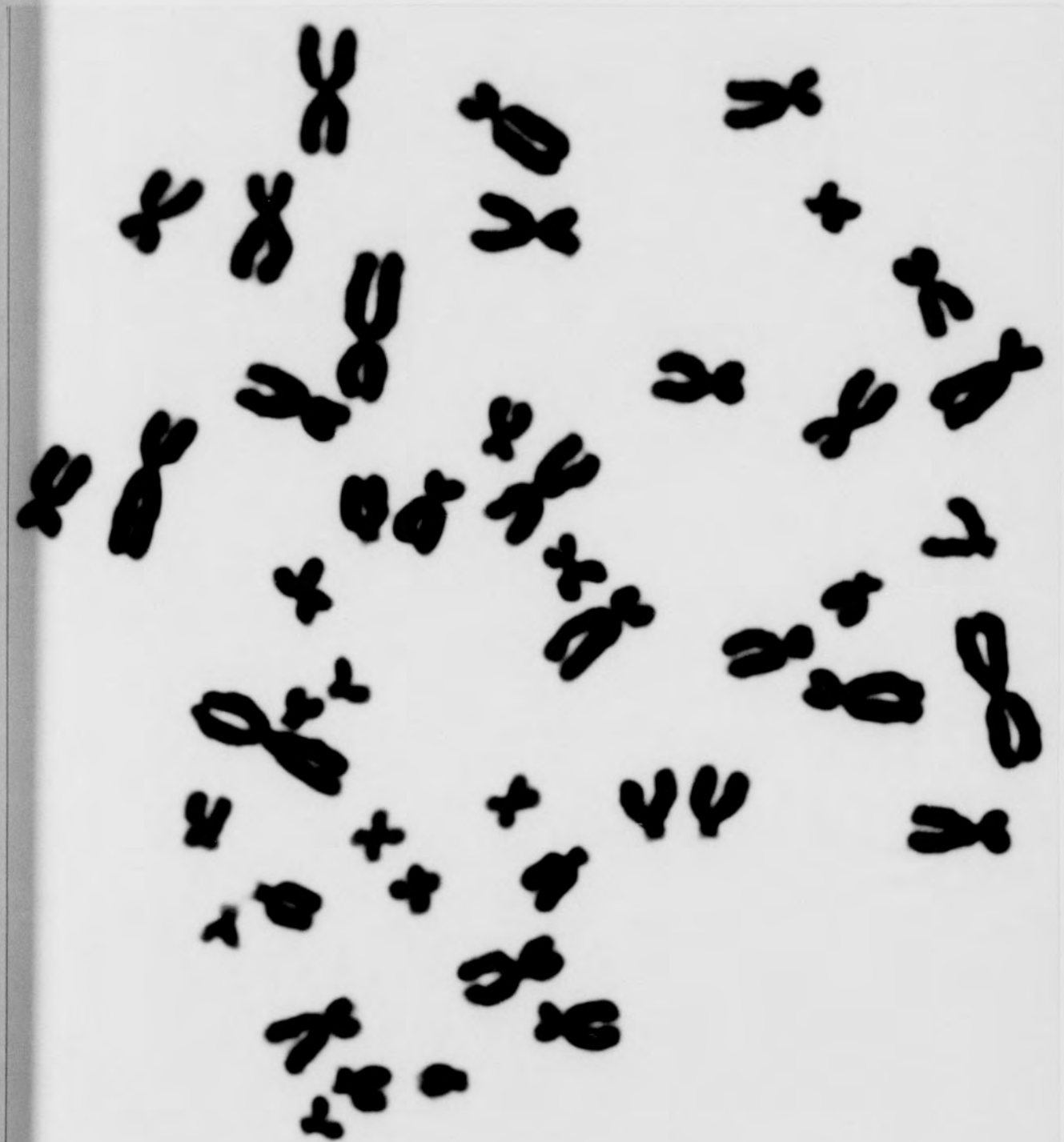


Figure 5. Karyotype of case no. 38

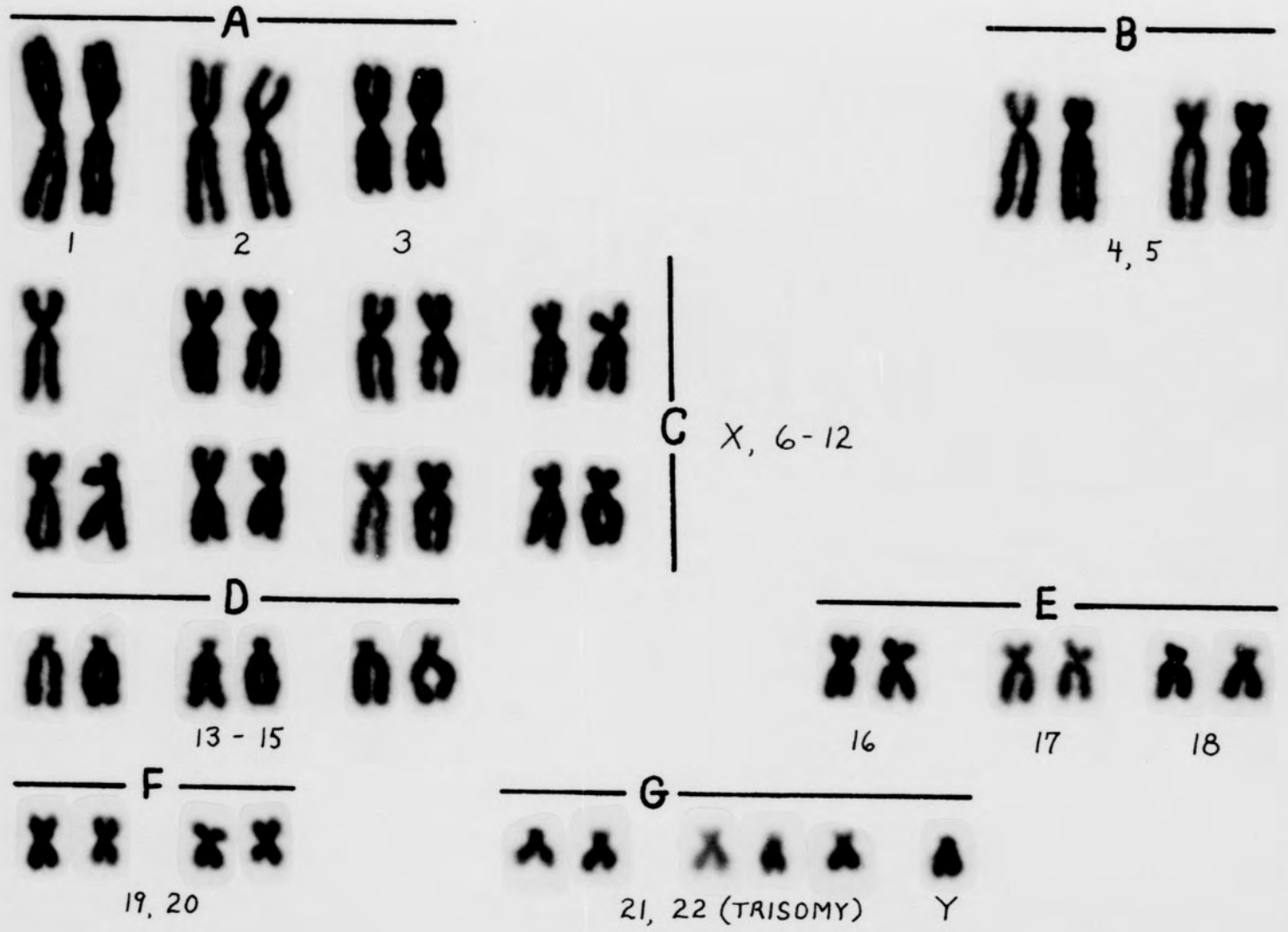

A
B
1
2
3
4, 5
C
X, 6-12
D
E
13 - 15
16
17
18
F
G
19, 20
21, 22 (TRISOMY)
Y

Figure 6. Metaphase chromosome spread of karyotype, Fig. 5

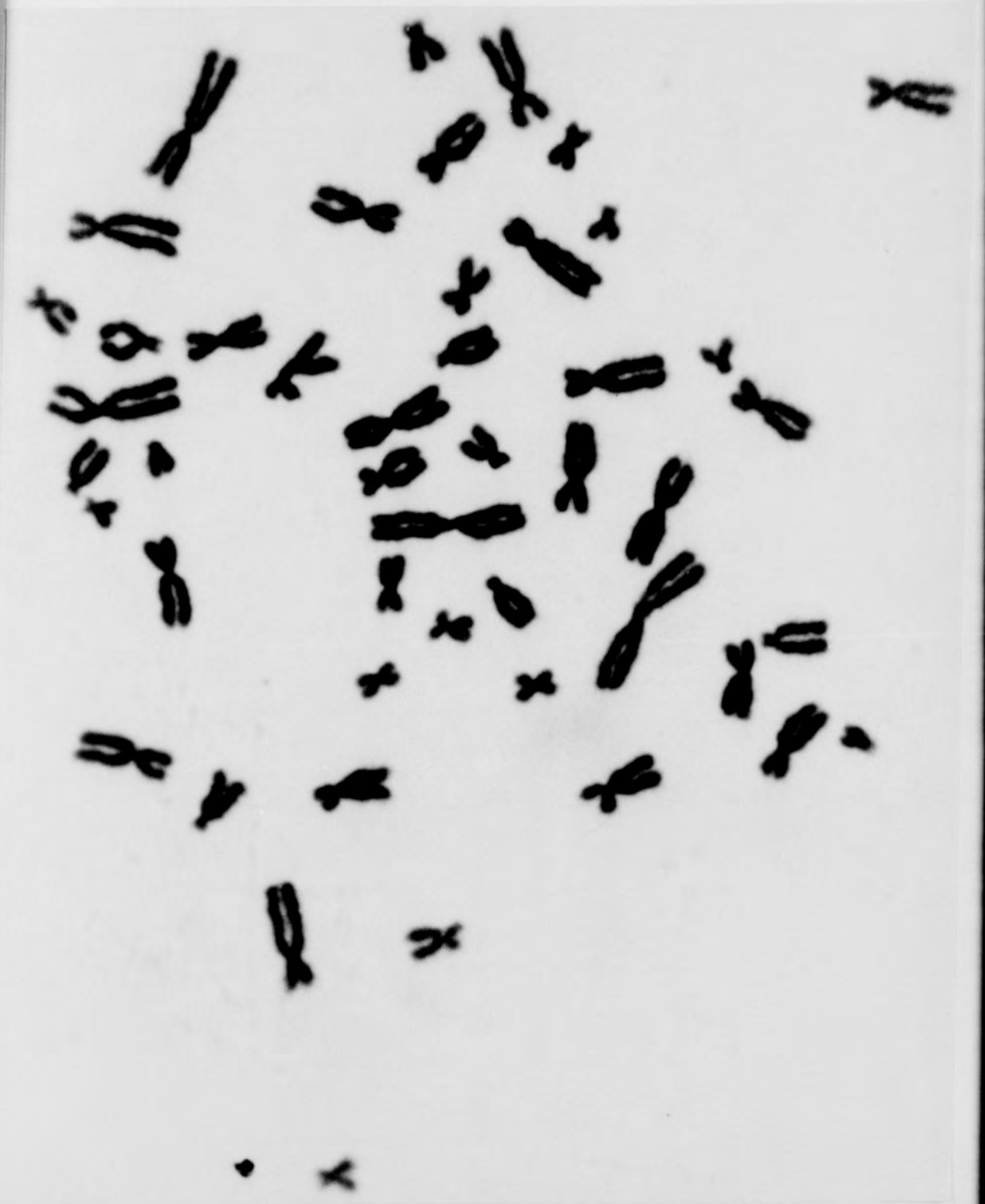


Fig. 5

Clinical Report 3

1. Case no.: 1 2. Age: 4½ months 3. Date of birth: 26 July 1965

4. Race: White-Indian 5. Sex: female 6. Date blood taken: 14 Dec. 1965

7. Age of mother at birth of propositus: 20

Present state of health: living and well; has asthma, but otherwise negative for familial diseases

8. Age of father at birth of propositus: 30

Present state of health: living and well

9. Siblings: none

10. Conceptual history: first pregnancy

11. History of pregnancy: no difficulties

12. Labor: spontaneous, hard, duration of 2 hr.

Delivery: premature

13. Birth weight: 3 lb. 10 oz. Length: 16½ inches

Head circumference: 12 inches Chest: 10 inches

Respiration: very sluggish

14. Physical examination and clinical history

Physical examination at birth revealed a long and narrow head, receding chin, mild tongue-tie, inability to close right eye, tight abductors of hips, flexion of fingers with bilateral curving of second and third fingers, and questionable clubbing of left foot. The infant was cyanotic and limp on arrival in the nursery, and was placed in oxygen and Alevaire with improvement. Subsequent examination revealed an obvious right facial weakness and tight clenching of fists. She had trouble both sucking and swallowing and had to be fed by gavage for the first month. She was

discharged at 8 weeks of age, weighing 5 lb. 15½ oz.

The infant was admitted to the hospital 3 weeks later with acute and chronic upper respiratory infections. The mother reported that the infant had been ill with a "cold" since she was discharged from the hospital. Examination revealed rhinitis, pharyngitis, acute otitis media, and conjunctivitis. Asymmetry of the face was also noted at this time. The infant responded to antibiotic therapy and was discharged a week later.

The last admission, at age 4½ months, was because of respiratory difficulty. On arrival in the emergency room the infant was cyanotic, apneic, cold, and listless. She was resuscitated there and was maintained on oxygen and croupette following tracheotomy. Physical examination at this time revealed a small, severely ill infant with multiple congenital anomalies, including small head with prominent occiput, small mandible, short sternum, low-set and flabby ears, crossing of index fingers over the third fingers, and bilateral calcuviovalgus. The severe respiratory distress was attributed to a relatively enlarged tongue. Edwards' syndrome was suspected. Her condition remained very poor. Immediately after blood for chromosome analysis was taken by heel puncture the infant ceased breathing, but she responded to resuscitation. Two days later she ceased breathing and turned cyanotic again; this time she did not respond to resuscitation, and death was attributed to respiratory failure and general inanition. Autopsy was not performed.

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells		3		33		36
% occurrence		8.3%		91.7%		

Karyotype	No. of chromosomes	Additional chromosome(s)	Missing chromosome(s)
No. 1	47	No. 18	
No. 2	47	No. 18	
No. 3	47	No. 18	
No. 4	47	No. 18	
No. 5	47	No. 18	
No. 6	47	No. 18	
No. 7	47	No. 18	
No. 8	47	No. 18	
No. 9	47	No. 18	
No. 10	47	No. 18	
No. 11	45	No. 18	No. 16, G
No. 12	45	No. 18	B, G

These findings were consistent with a diagnosis of Edwards' syndrome, or trisomy 18 syndrome (Fig. 7, page 29).

Figure 7. Karyotype of case no. 1

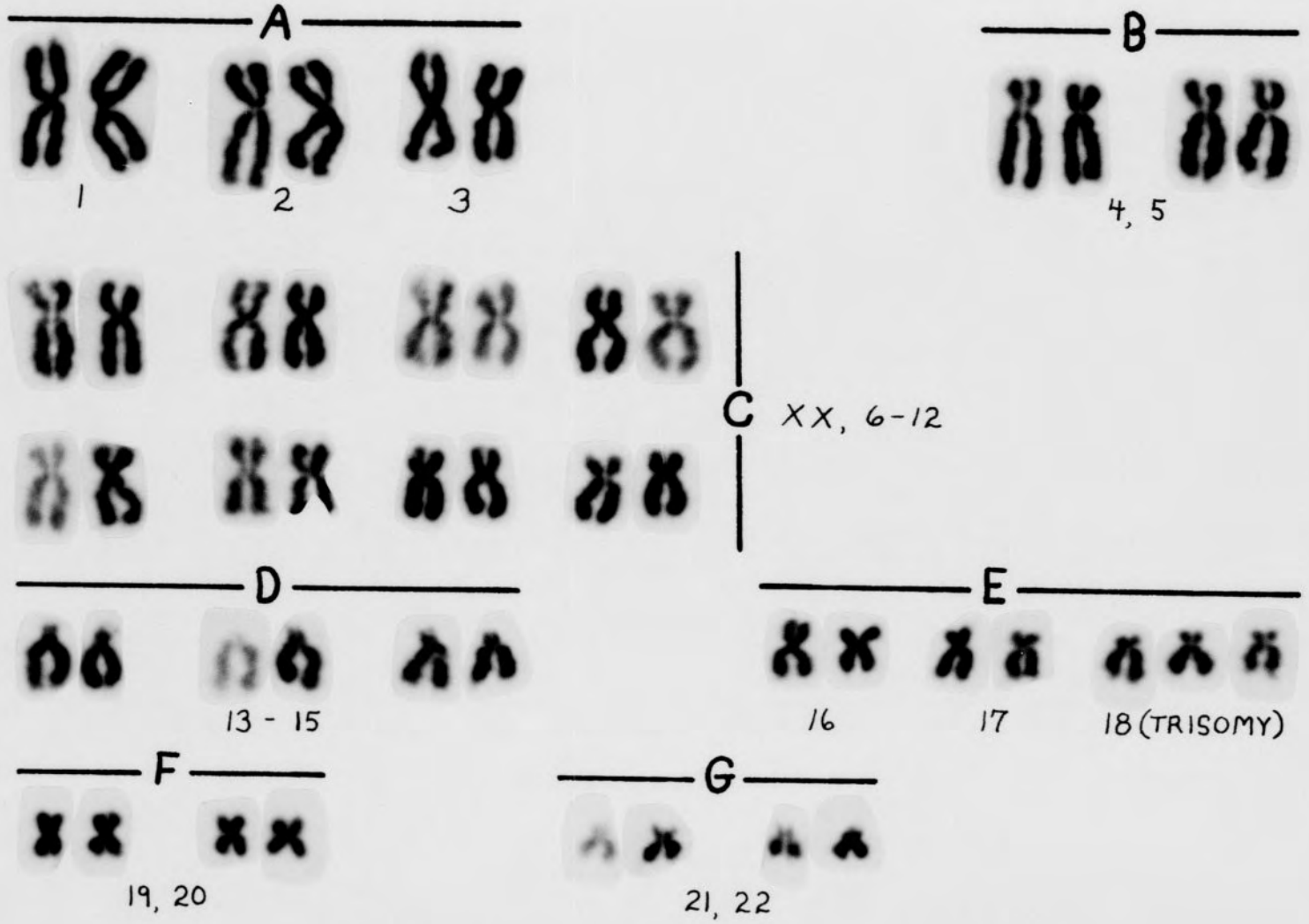
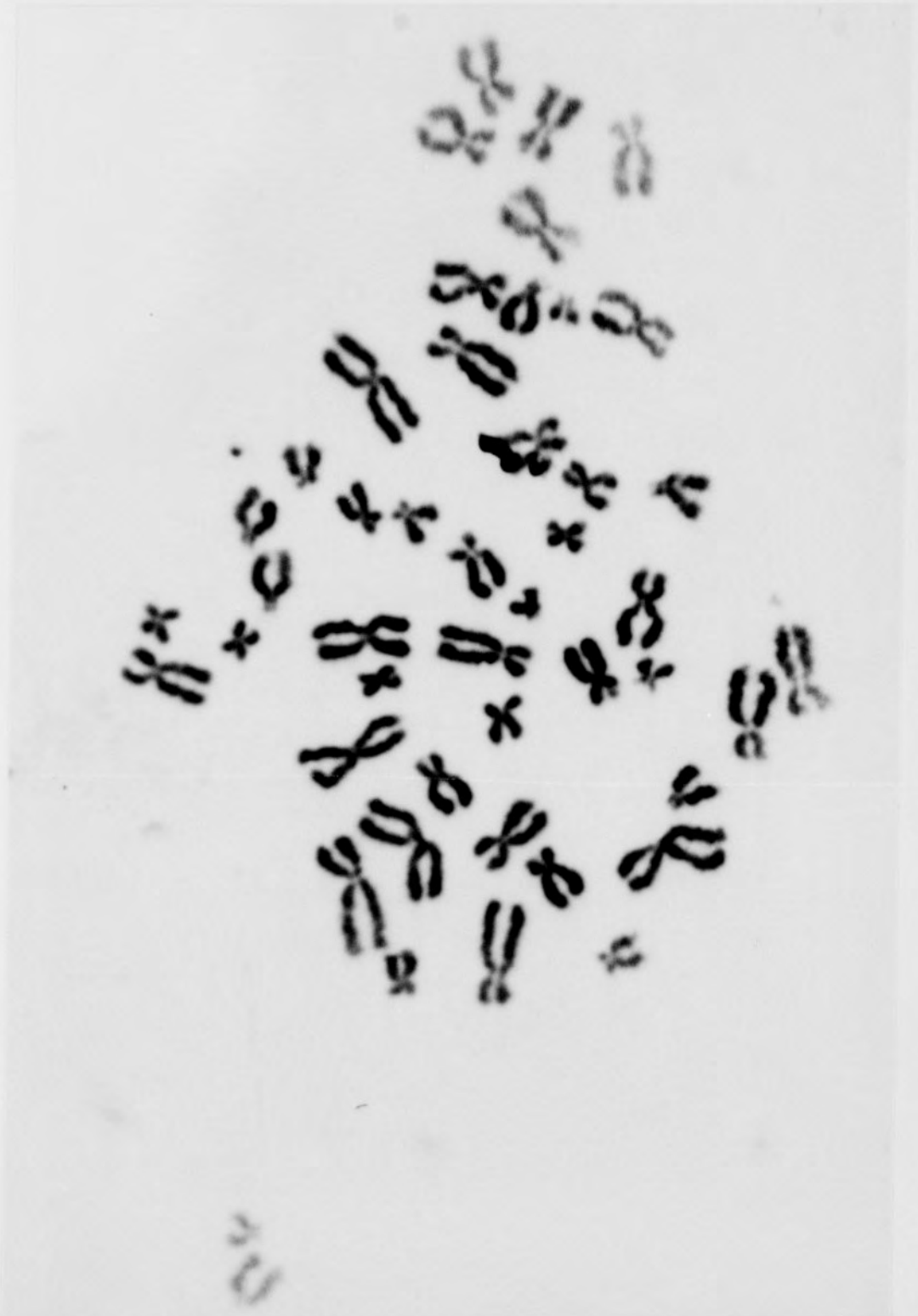

A
B
1
2
3
4, 5
C XX, 6-12
D
E
13 - 15
16
17
18 (TRISOMY)
F
G
19, 20
21, 22

Figure 8. Metaphase chromosome spread of karyotype, Fig. 7

e, Fig. 7



Clinical Report 4

1. Case no.: 45 2. Age: 5 days 3. Date of birth: 16 Nov. 1968

4. Race: White 5. Sex: female 6. Date blood taken: 21 Nov. 1968

7. Age of mother at birth of propositus: 40

Present state of health: N. R.

8. Age of father at birth of propositus: N. R.

Present state of health: N. R.

9. Siblings: none

10. Conceptual history: N. R.

11. History of pregnancy: N. R.

12. Labor: N. R.

Delivery: term, spontaneous, frank breech

13. Birth weight: 4 lb. 15½ oz. Length: 19 inches

Head circumference: 12¾ inches Chest: 11-3/4 inches

Respiration: difficult

14. Physical examination and clinical history

Physical examination at birth revealed a very listless infant who cried poorly and only with stimulation. It appeared to have right facial weakness, which may only have reflected asymmetry of the face; the right face was smaller with microphthalmia and ptosis. There was a hemangioma of the right lower leg, about the size of a quarter. The lungs were aerated well, but respirations were rapid. The placenta was small and fibrotic. The infant was admitted to the nursery cold and grunting and pale. It was placed in isolette with oxygen and humidity because of mild atelectasis.

The writer, when taking blood for chromosome analysis,

observed tight clenching of the fists with the thumb under the fingers and the index fingers crossed over the third fingers, small mandible, prominent occiput, low-set and malformed ears, and rocker bottom feet.

The infant expired at age 4½ months. Autopsy revealed emaciation with clinical failure to thrive. Gross findings included, in addition to the phenotypic abnormalities noted premortem by the physician and the writer, cyanosis. The lungs showed focal pulmonary hemorrhage. The heart showed interventricular septal defect, patent ductus arteriosus, and patent foramen ovale. The digestive system showed Meckel's diverticulum. Urogenital and other systems were not remarkable.

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells			3	37		40
% occurrence			7.5%	92.5%		

Karyotype	No. of chromosomes	Additional chromosome(s)	Missing chromosome(s)
No. 1	47	No. 18	
No. 2	47	No. 18	
No. 3	47	No. 18	
No. 4	47	No. 18	
No. 5	47	No. 18	
No. 6	47	No. 18	
No. 7	46	No. 18	No. 3
No. 8	46	No. 18	No. 16
No. 9	46	No. 18	No. 16

These findings were consistent with a diagnosis of Edwards' syndrome, or trisomy 18 syndrome (Fig. 9, page 36).

Figure 9. Karyotype of case no. 45

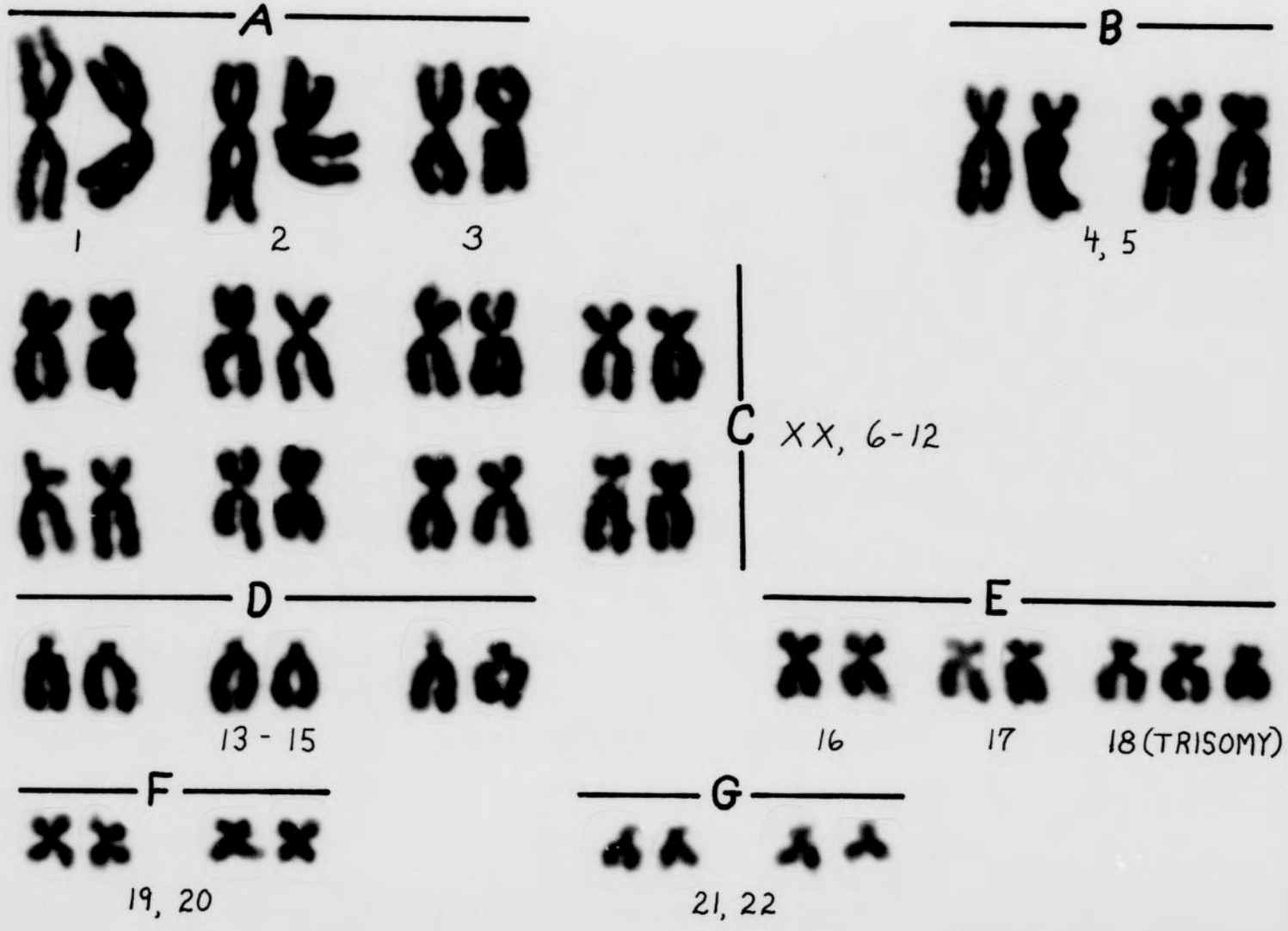

A
B
1
2
3
4, 5
C XX, 6-12
D
E
13 - 15
16
17
18 (TRISOMY)
F
G
19, 20
21, 22

Figure 10. Metaphase chromosome spread of karyotype, Fig. 9

Fig. 9

Clinical Report 5

1. Case no.: 29 2. Age: 8 weeks 3. Date of birth: 14 Dec. 1967

4. Race: Negro 5. Sex: female 6. Date blood taken: 12 Feb. 1968

7. Age of mother at birth of propositus: 19

   Present state of health: living and well

8. Age of father at birth of propositus: 22

   Present state of health: living and well

9. Siblings: none

10. Conceptual history: first pregnancy

11. History of pregnancy: N. R.

12. Labor: normal

    Delivery: term, spontaneous, normal

13. Birth weight: 5 lb. 2 oz. Length: N. R.

    Head circumference: N. R. Chest: N. R.

    Respiration: difficult

14. Physical examination and clinical history

    Physical examination at birth revealed non-pitting edema of the feet, ankles, and lower legs, and of the right eyelid. Bilirubin rose to over 12 with a subsequent drop to normal.

    The first hospital admission, at the age of 1 month, was because of respiratory difficulty and markedly diminished appetite. Physical examination revealed a frail, lethargic, weak, pale infant in acute respiratory distress. Weight was 6 lb. 7½ oz., length 20 inches, head 14 inches. The edema present at birth had persisted. A heart murmur was noted at this time. The infant had a weak suck and weak palmar and plantar grasp. Laboratory findings revealed acidosis and

hyperkalemia, and chest X-ray revealed increased heart size. Buccal smears were negative for sex chromatin (none in over 300 cells and none in 200 cells). The patient responded well to iv fluids with bicarbonate and to digitalization. The diagnosis was gonadal dysplasia and congenital heart disease, incompletely diagnosed.

The second hospital admission, at the age of 3 months, was because of complete loss of appetite 1½ days prior to admission and progressive lethargy. Physical examination revealed a pale, flaccid, lethargic, edematous infant with rapid and shallow respirations. The infant was treated with adrenal hormones and iv fluids. While being observed by the attending pediatrician and an intern, the patient suffered a cardio-respiratory arrest. There was no response to immediate external cardiac massage and resuscitation.

Autopsy findings included fibrous thickening of the linings of the left ventricle and left atrium, pericardial effusion, myocardial hypertrophy, ductus, and dilatation of the pulmonary trunk (approximately half again as big as the aorta). Fibrous tissue was found representing ovarian tissue; microscopic examination of multiple sections failed to show any follicles. The morphology of the uterus was normal.

15. Cytogenetic findings

Chromosome counts	43	44	45	46	47	Total cells counted
No. of cells	3	2	25			30
% occurrence	10.0%	6.7%	83.3%			

Eight karyotypes with 45 chromosomes had only 15 chromosomes in group C, presumably lacking an X chromosome (Fig. 11, page 43). These findings were consistent with a diagnosis of gonadal dysgenesis, or Turner's syndrome.

Of the 30 cells examined, five (16.7%) contained at least one chromosome with a gap, and two (6.7%) contained a chromosome break.

Figure 11. Karyotype of case no. 29

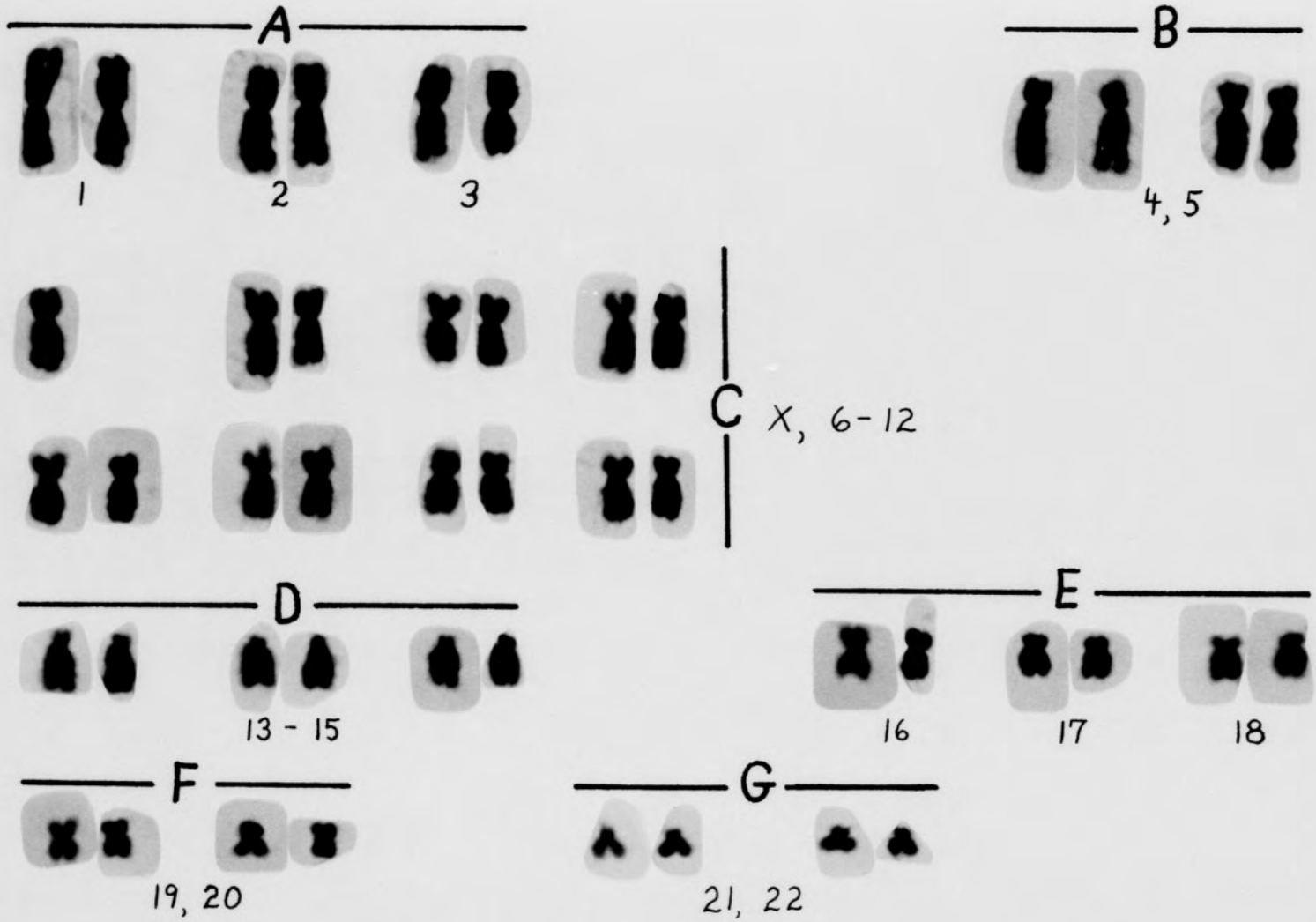

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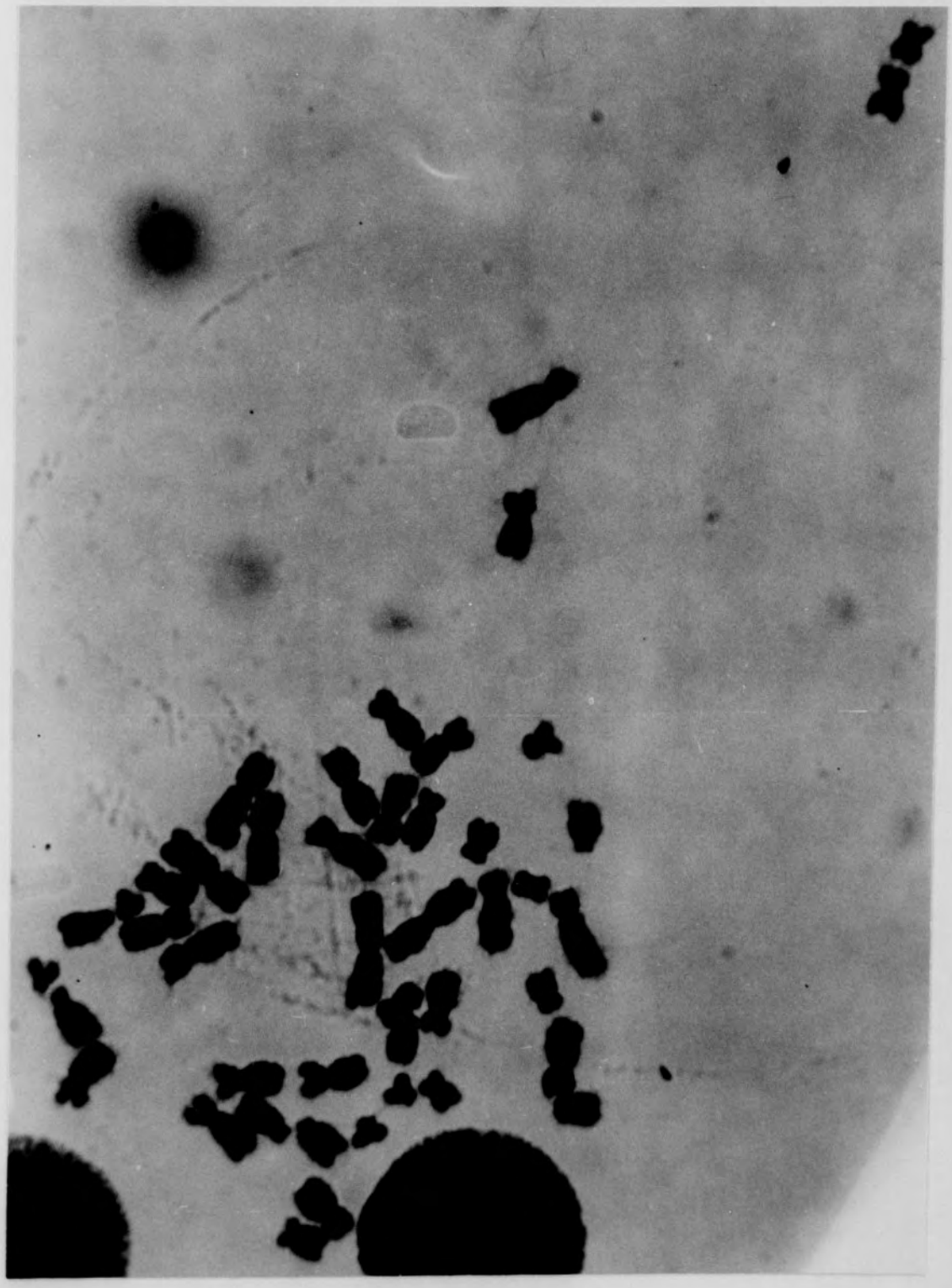
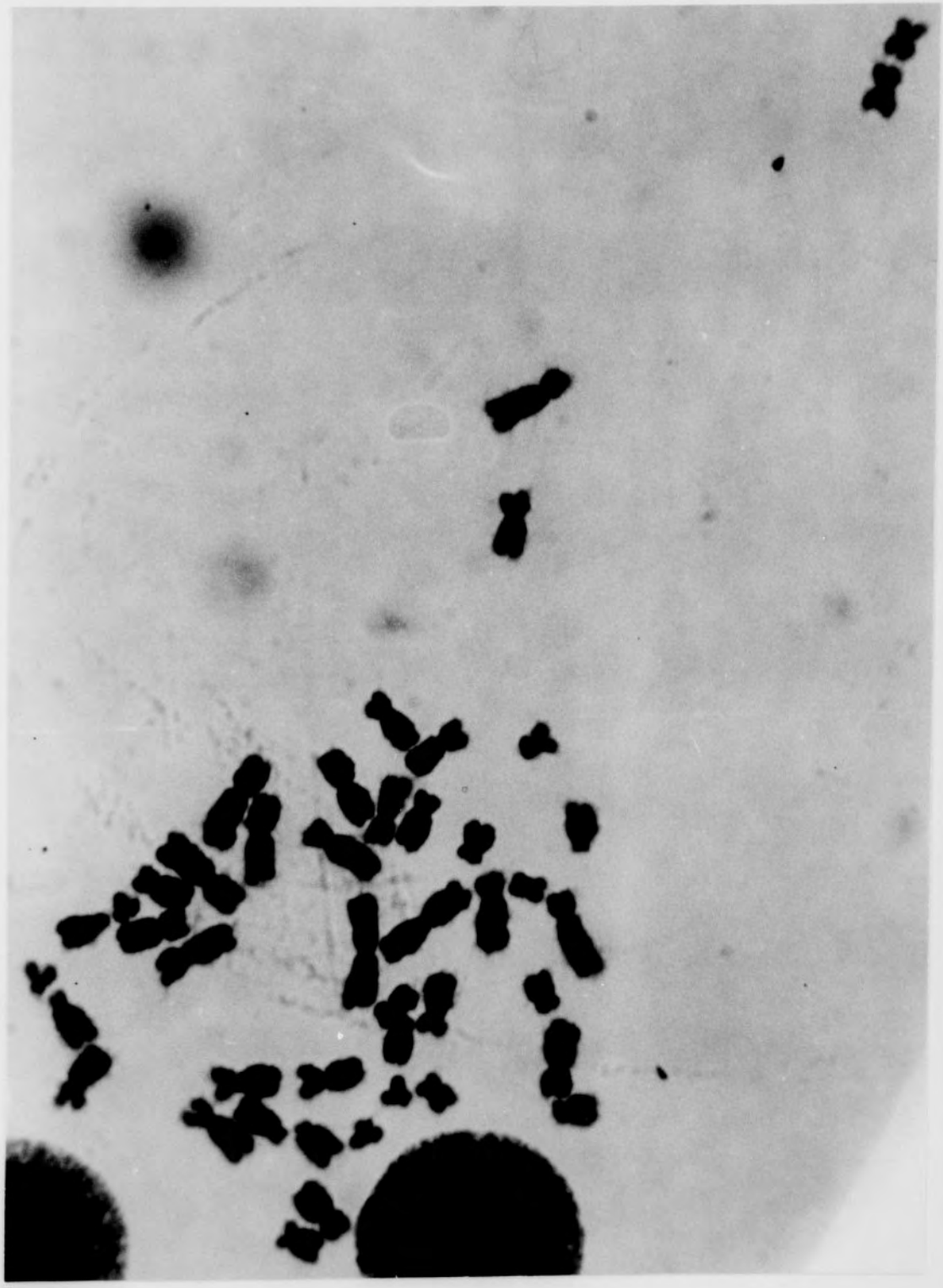
Figure 12. Metaphase chromosome spread of karyotype, Fig. 11

pe, Fig. 11



pe, Fig. 11



Clinical Report 6

1. Case no.: 8 2. Age: 1 month 3. Date of birth: 7 June 1966

4. Race: White 5. Sex: male 6. Date blood taken: 7 July 1966

7. Age of mother at birth of propositus: 19

Present state of health: good except for slight obesity and occasional tonsillitis

8. Age of father at birth of propositus: N. R.

Present state of health: N. R.

9. Siblings: N. R.

10. Conceptual history: N. R.

11. History of pregnancy: N. R.

12. Labor: 8 hr.

Delivery: term, outlet forceps

13. Birth weight: 8 lb. 1 oz. Length: 20¼ inches

Head circumference: N. R. Chest: N. R.

Respiration: good

14. Physical examination and clinical history

Physical examination shortly after birth revealed a healthy baby; no defects were noted.

A second examination at age 9 days showed the infant to be in excellent physical condition, and it was admitted to the Children's Home for adoption.

A routine examination by a physician at the Children's Home revealed that the infant's penis, although normal, was embedded in a suprapelvic fat pad; both testicles were in the scrotum. A buccal smear at age 2 weeks revealed that 30% of the nuclei were sex-chromatin positive.

15. Cytogenetic findings

Peripheral blood smear for sex chromatin - 12 of 100 polymorphonuclear neutrophils were sex-chromatin positive, each containing a single drumstick (Fig. 13, page 49).

Buccal smear for sex chromatin - 34 of 100 buccal cells were sex-chromatin positive, each containing a single Barr body (Fig. 14, page 51).

Chromosome counts	44	45	46	47	48	Poly-ploid	Total cells counted
No. of cells		5	6	38		1	50
% occurrence		10%	12%	76%		2%	

Karyotype	No. of chromosomes	Additional chromosome(s)	Missing chromosome(s)
No. 1	47	X	
No. 2	47	X	
No. 3	47	X	
No. 4	47	X	
No. 5	47	X	
No. 6	47	X	
No. 7	47	X	
No. 8	46	X	No. 16
No. 9	46	X	No. 16
No. 10	46	X	No. 18
No. 11	46	X	F
No. 12	45	X	D, F
No. 13	45		F

Note the inconsistent loss of chromosome(s) in the cells with fewer than 47 chromosomes. These findings were consistent with a diagnosis of Klinefelter's syndrome, having a sex chromosome complement XXY (Fig. 15, page 53).

Figure 13. Polymorphonuclear neutrophil with sex-chromatin appendage, case no. 8

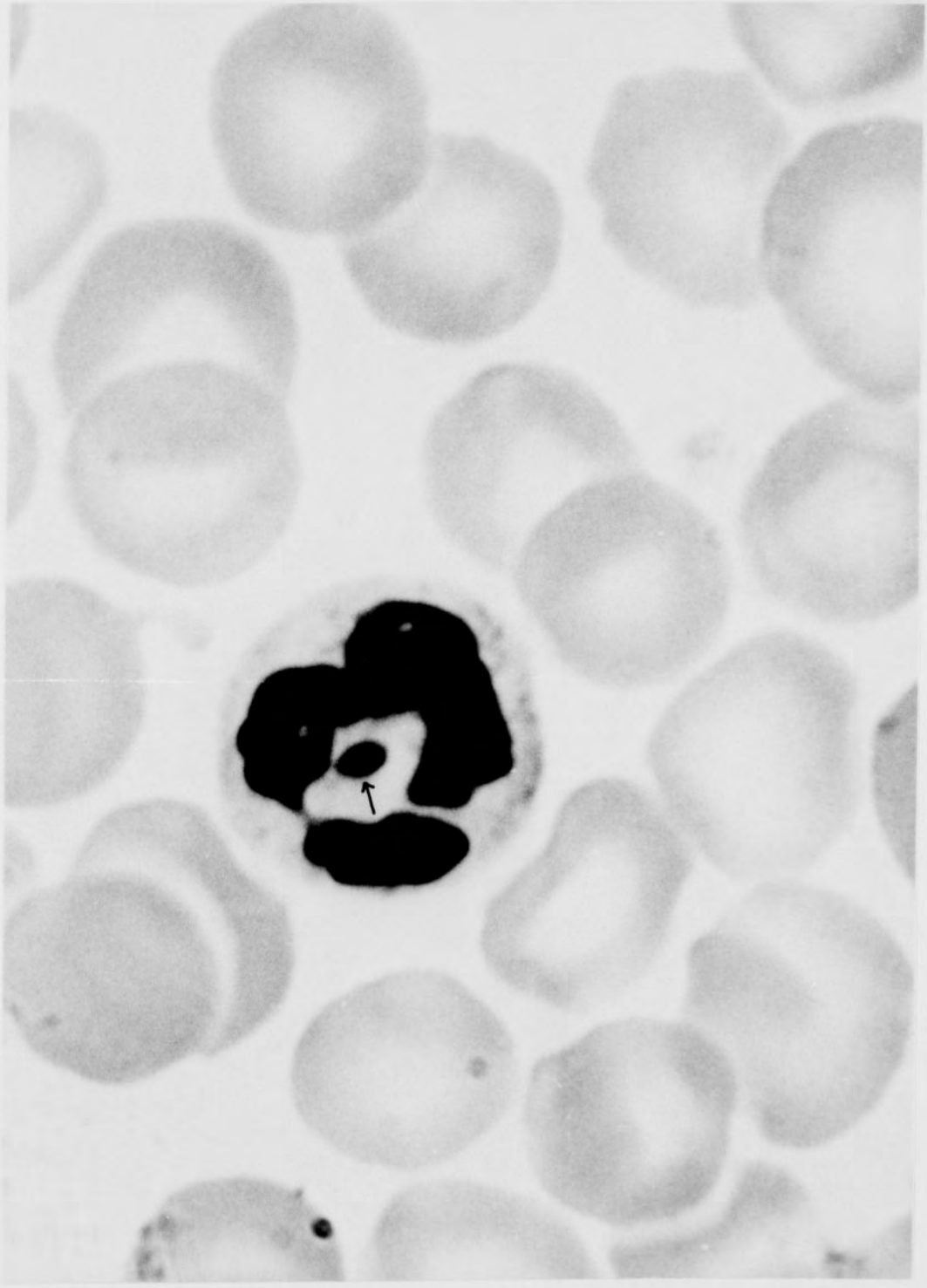


Figure 14. Buccal cell with sex-chromatin mass (Barr body), case no. 8

(Barr body),

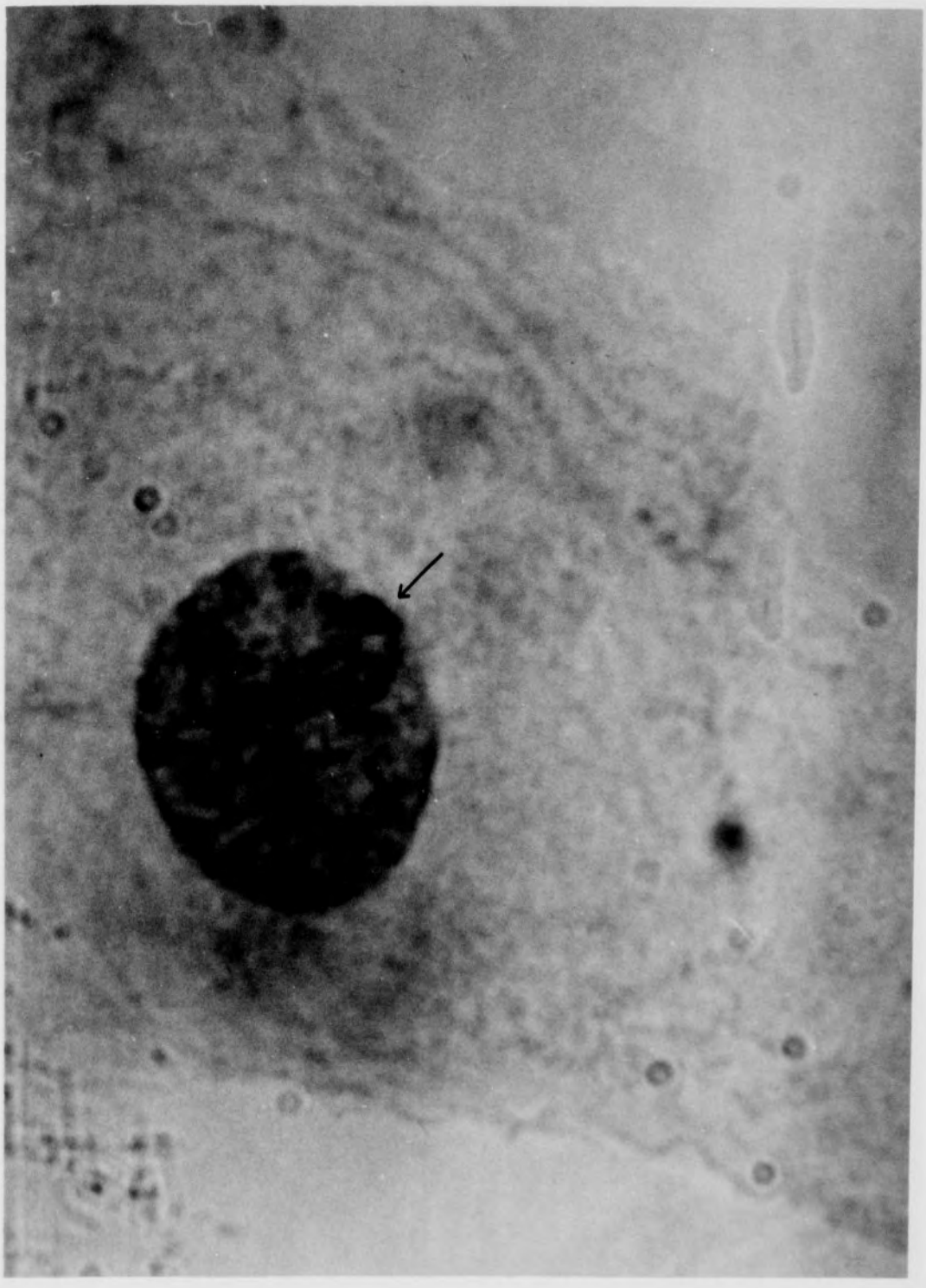


Figure 15. Karyotype of case no. 8

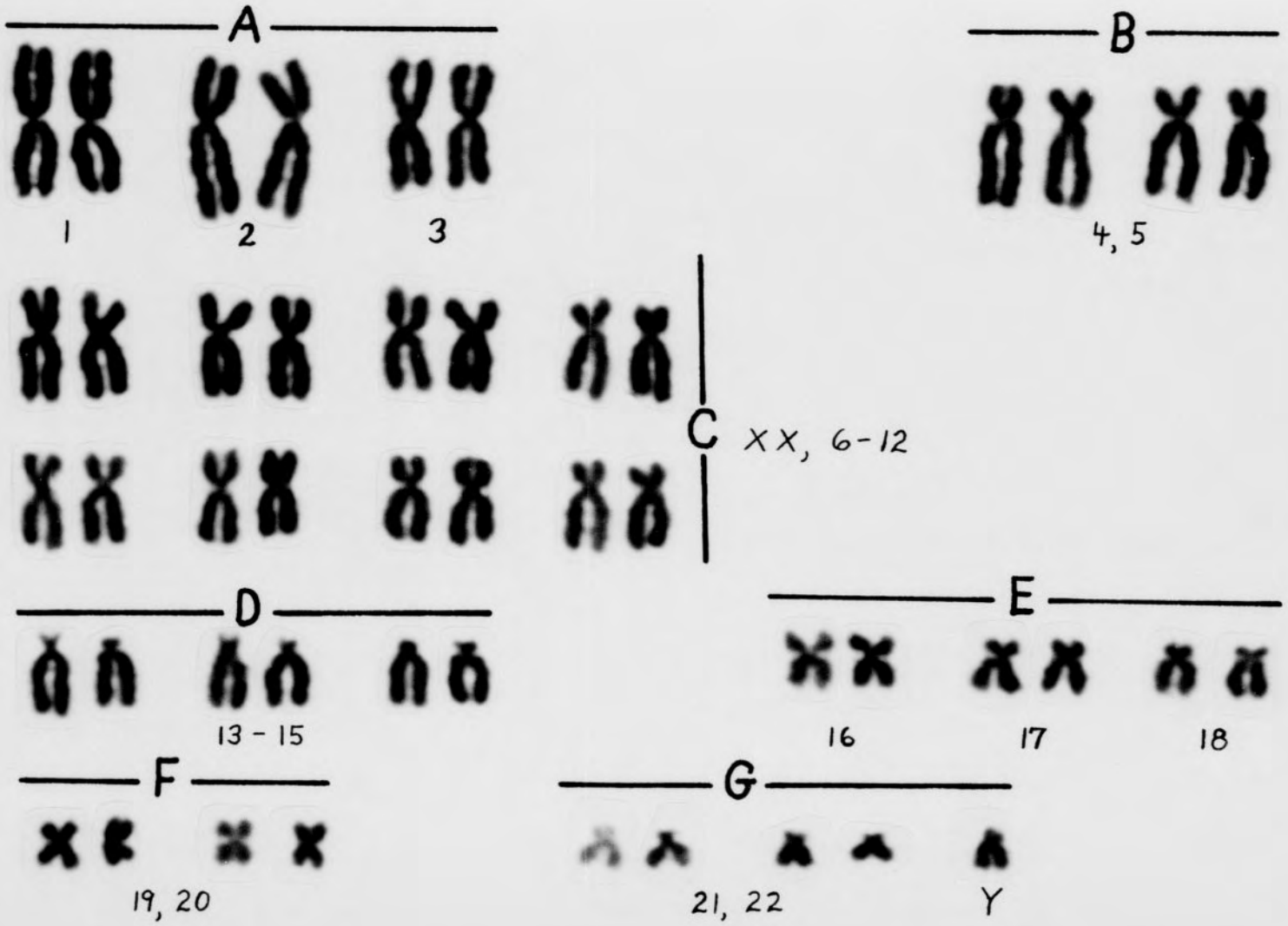

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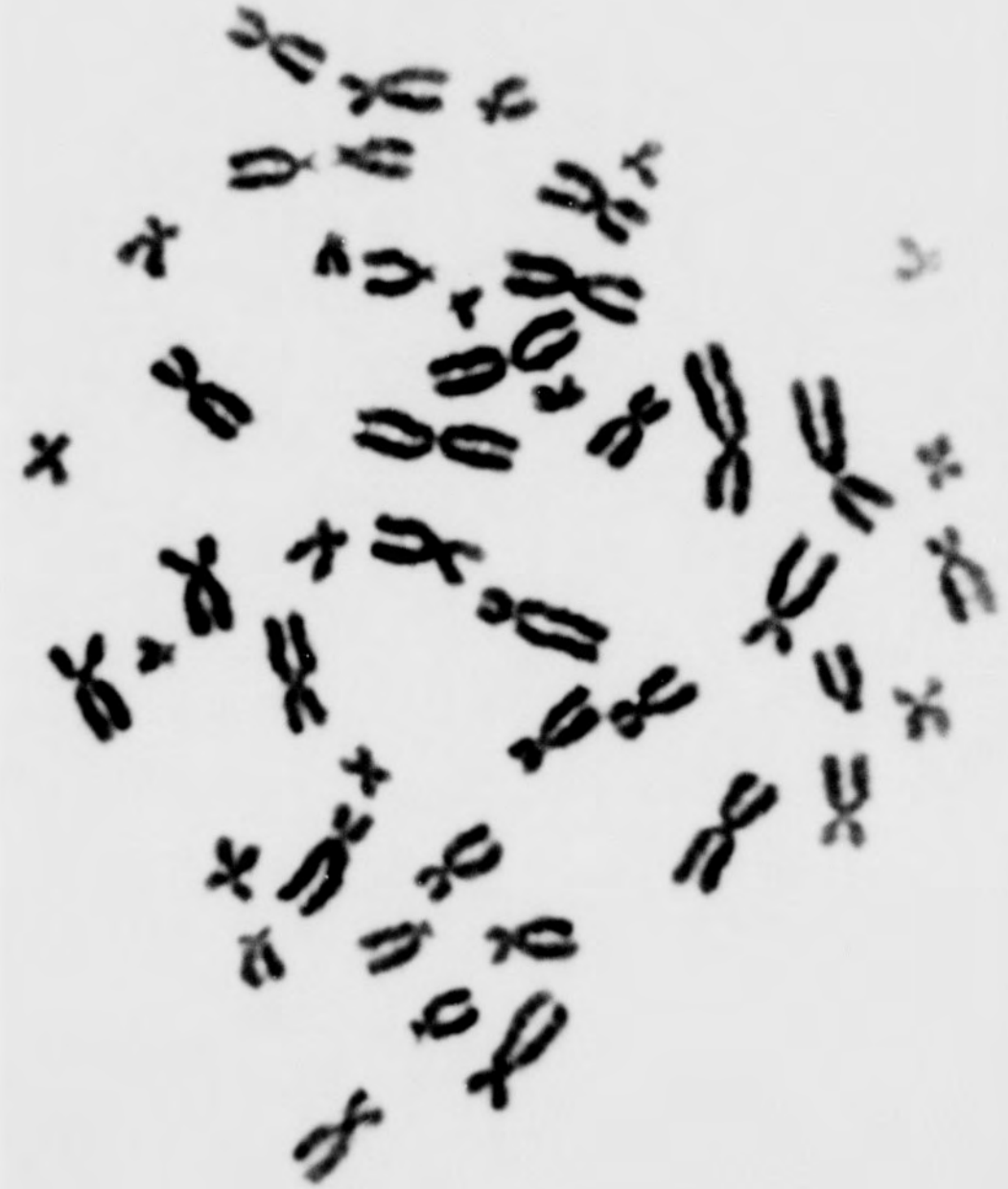
Figure 16. Metaphase chromosome spread of karyotype, Fig. 15



Clinical Report 7

1. Case no.: 30 2. Age: 13½ 3. Date of birth: 14 July 1954

4. Race: White 5. Sex: female 6. Date blood taken: 22 Feb. 1968

7. Age of mother at birth of propositus: 27

   Present state of health: good; sensitive to drugs and anesthesia, and has had many blood transfusions

8. Age of father at birth of propositus: 31

   Present state of health: good

9. Siblings: brother, age 20, fair health (allergies, anemia, many colds); sister, age 15, good health; brother, age 12, heart murmur and allergies; brother, age 10, with heart condition, episodes of stoppage of breathing, and allergies; sister, age 7, good health except for allergies; brother, age 8 months, good health

10. Conceptual history: four additional pregnancies resulted in abortions with hemorrhage

11. History of pregnancy: took male hormones by injection and by mouth, and took medications for losing weight (fluid) and to suppress appetite; salt-free diet; lost 1/2 lb. during pregnancy

12. Labor: "forced"

    Delivery: premature, 7 months gestation

13. Birth weight: 5 lb. 1 oz. Length: N. R.

    Head circumference: N. R. Chest: N. R.

    Respiration: difficult

14. Physical examination and clinical history

Physical examination at age 13½ years revealed a tall and slender female with no secondary sex characteristics and with epicanthal folds. The patient is considered to be moderately retarded; she especially has difficulty with numbers. She has eczema and is allergic to cow's milk. Muscular coordination is fairly good; the muscles are decreased in size, but strength is normal. The patient tired easily as a child. The extremities are quite long, but the fingers and toes are not unusually long. Joint motion is normal.

Laboratory findings included normal electrocardiogram and electroencephalogram. Blood chemistries (SMA - 12) were normal for her age. Normal amounts of 17-ketosteroids and 17-ketogenic steroids were found in a 24-hour urine collection.

A skull film was normal, but a lateral film of the chest revealed pectus excavatum, which accounts for the posterior position of the heart and apparent increase in transverse diameter of the heart.

Later X-rays of the long bones revealed essentially normal bone and joint structure. The bones are rather long, but texture is normal and the bone age approximates the chronological age of the patient.

The onset of menses occurred at age 14 and appeared to be normal in amount and duration.

15. Cytogenetic findings

Peripheral blood smear for sex chromatin - four of 1000 polymorphonuclear neutrophils (0.4%) were sex-chromatin positive, each containing a single drumstick.

Buccal smears for sex chromatin

No. of Barr bodies	0	1	2	3	Total cells
Left cheek	357	101	33	9	500
Right cheek	379	75	33	13	500
Total	736	176	66	22	1000
% occurrence	73.6%	17.6%	6.6%	2.2%	

See Figures 17 to 20, pages 60 to 66.

Chromosome counts	44	45	46	47	48	49	Total cells counted
No. of cells	3	8	4	23	160	2	200
% occurrence	1.5%	4.0%	2.0%	11.5%	80.0%	1.0%	

Figure 17. Buccal cells with no Barr bodies, case no. 30

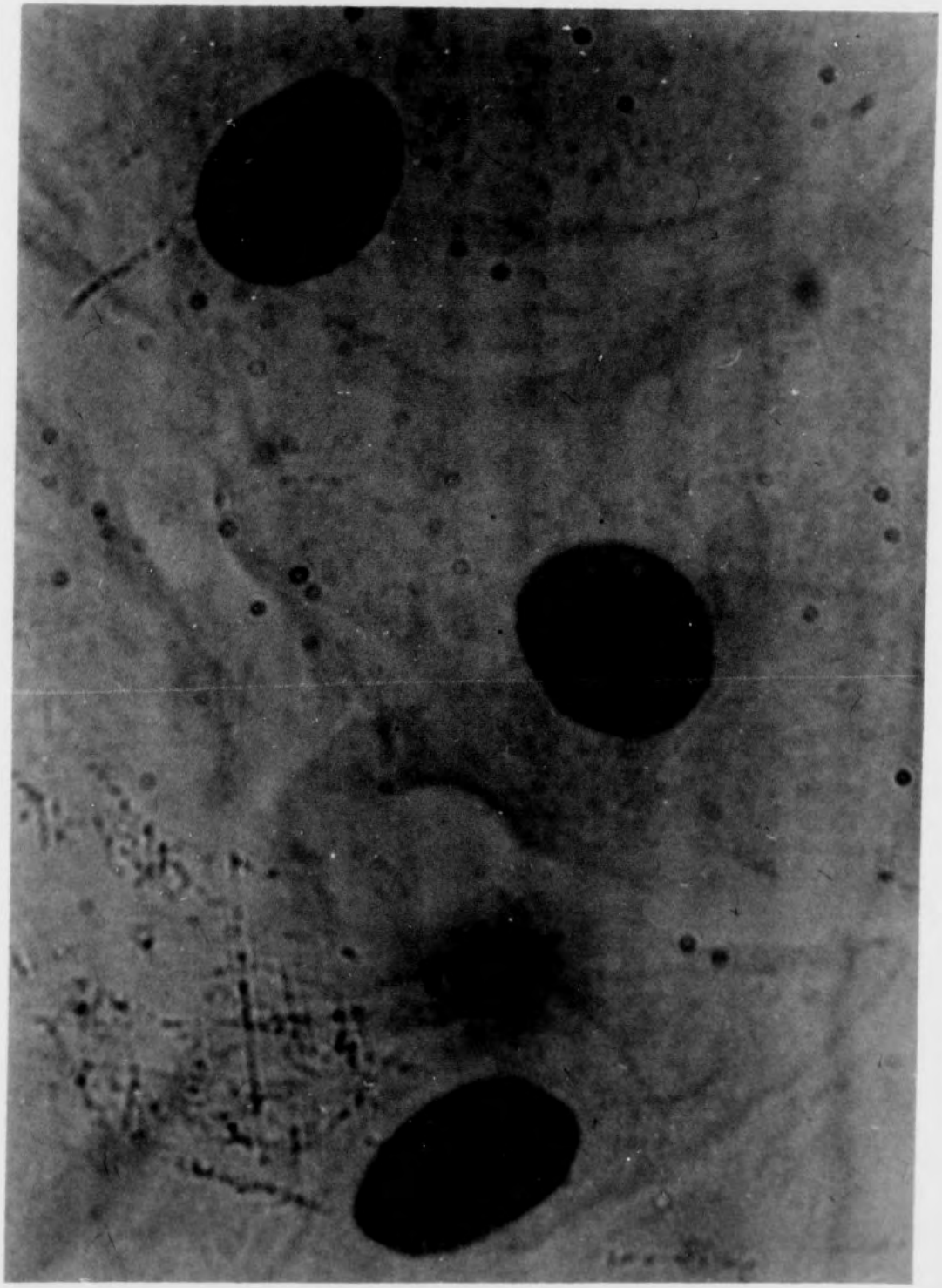


Figure 18. Buccal cell with one Barr body, case no. 30

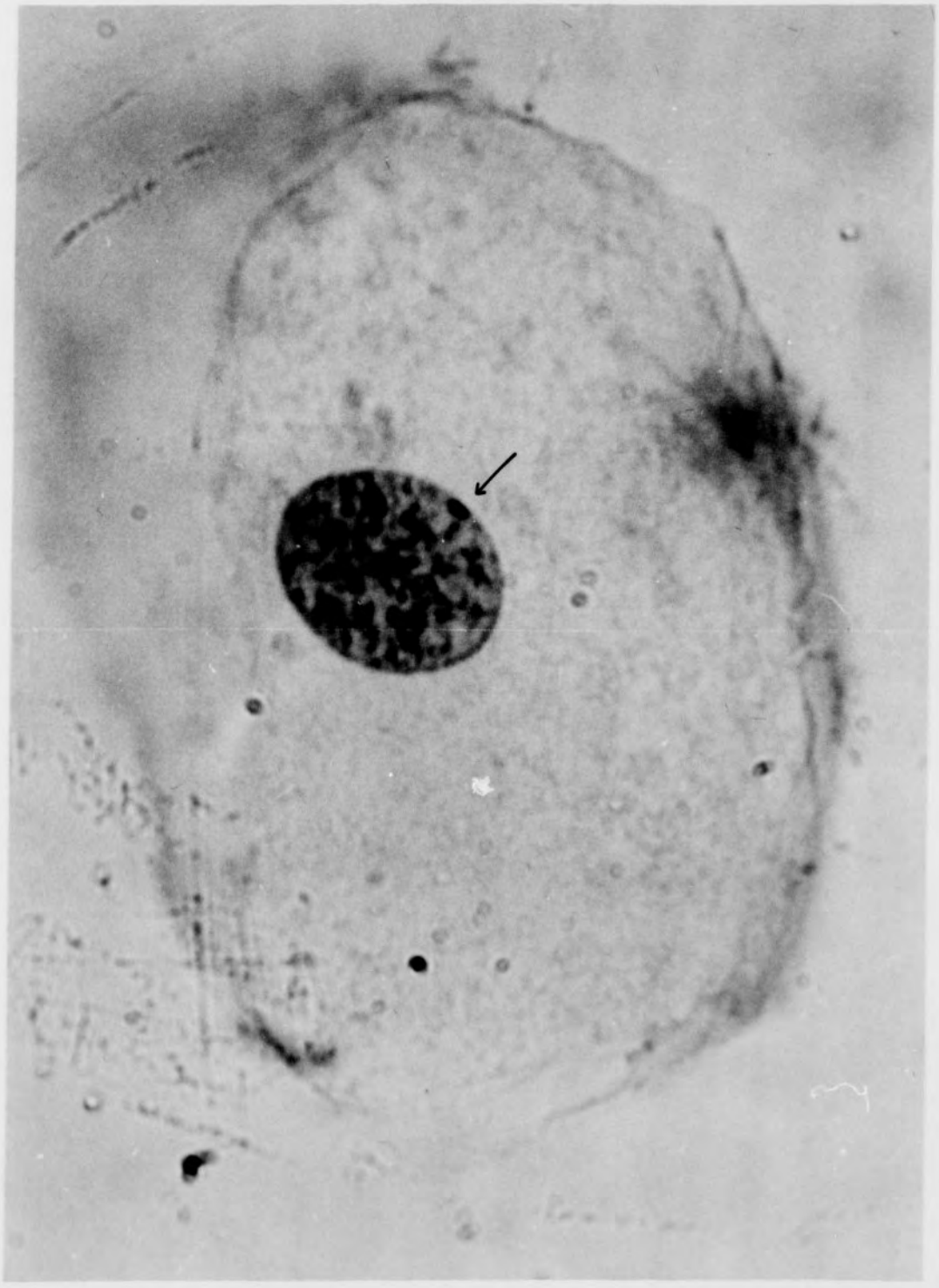
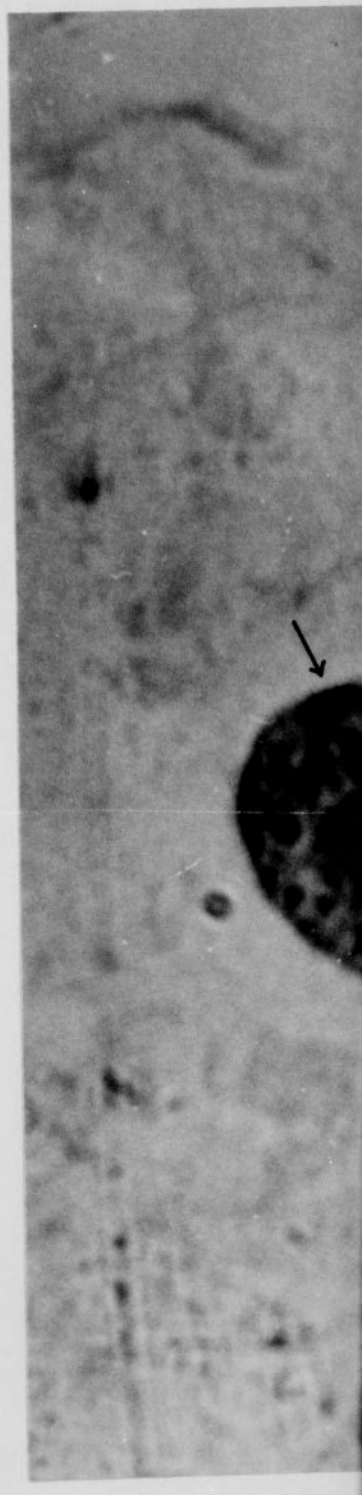


Figure 19. Buccal cell with two Barr bodies, case no. 30



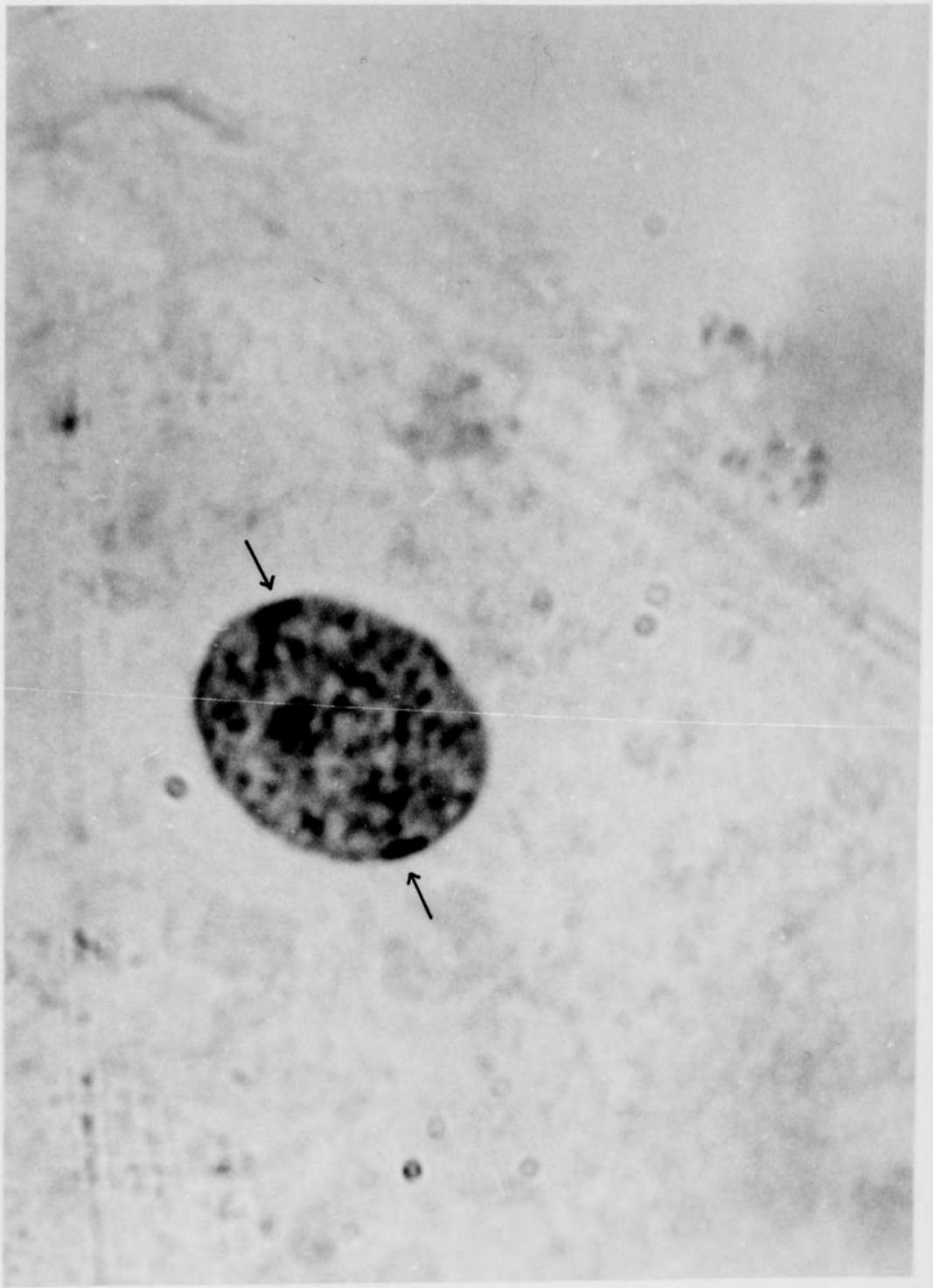
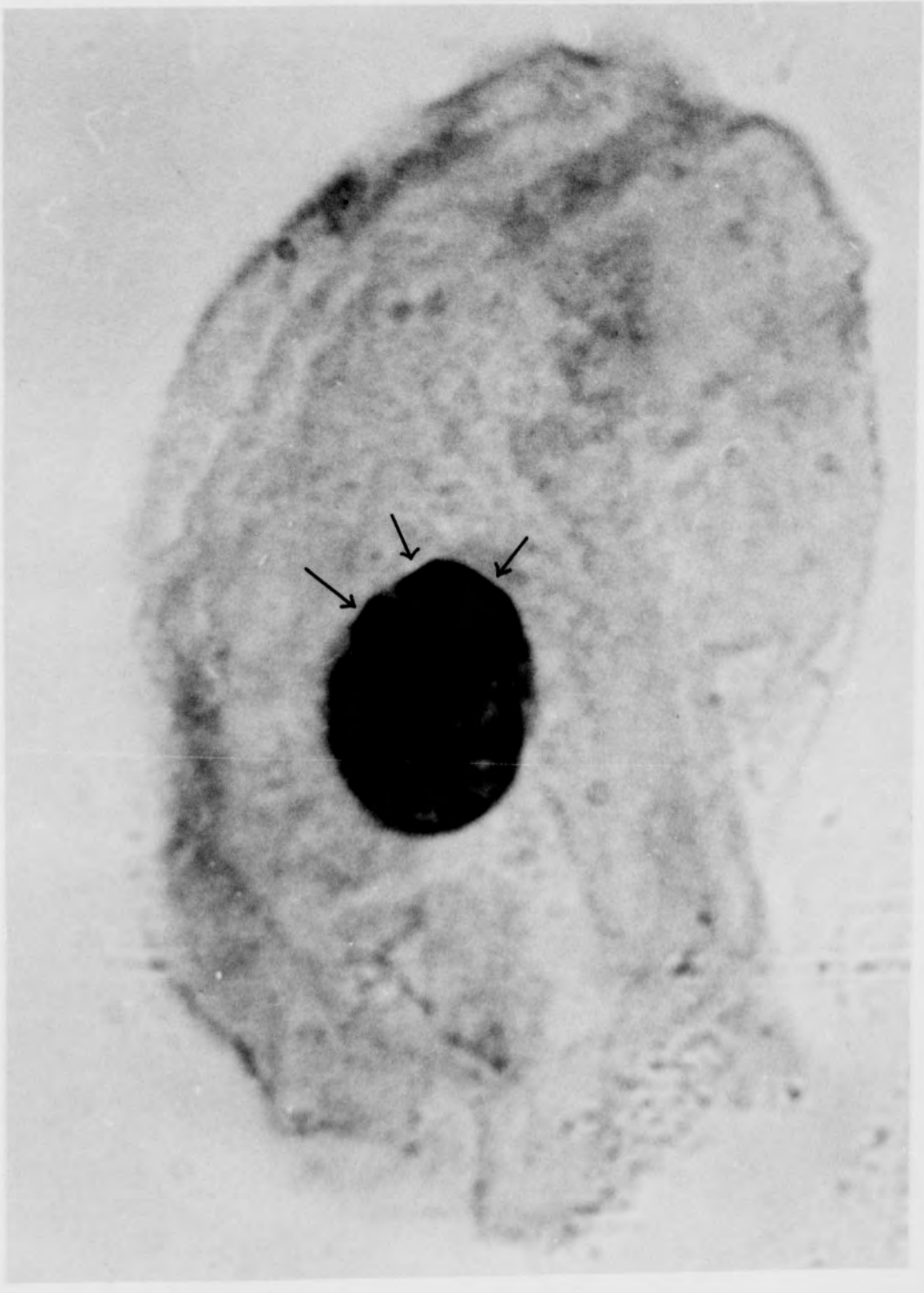


Figure 20. Buccal cell with three Barr bodies, case no. 30

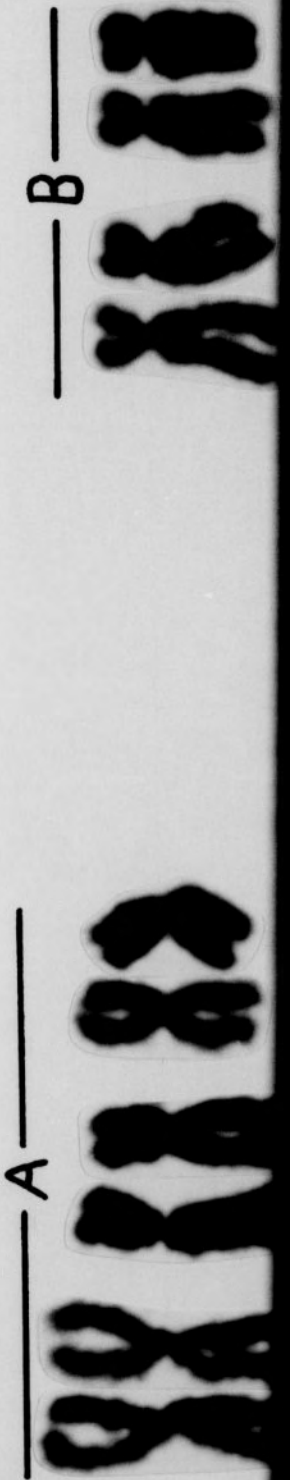
se no. 30

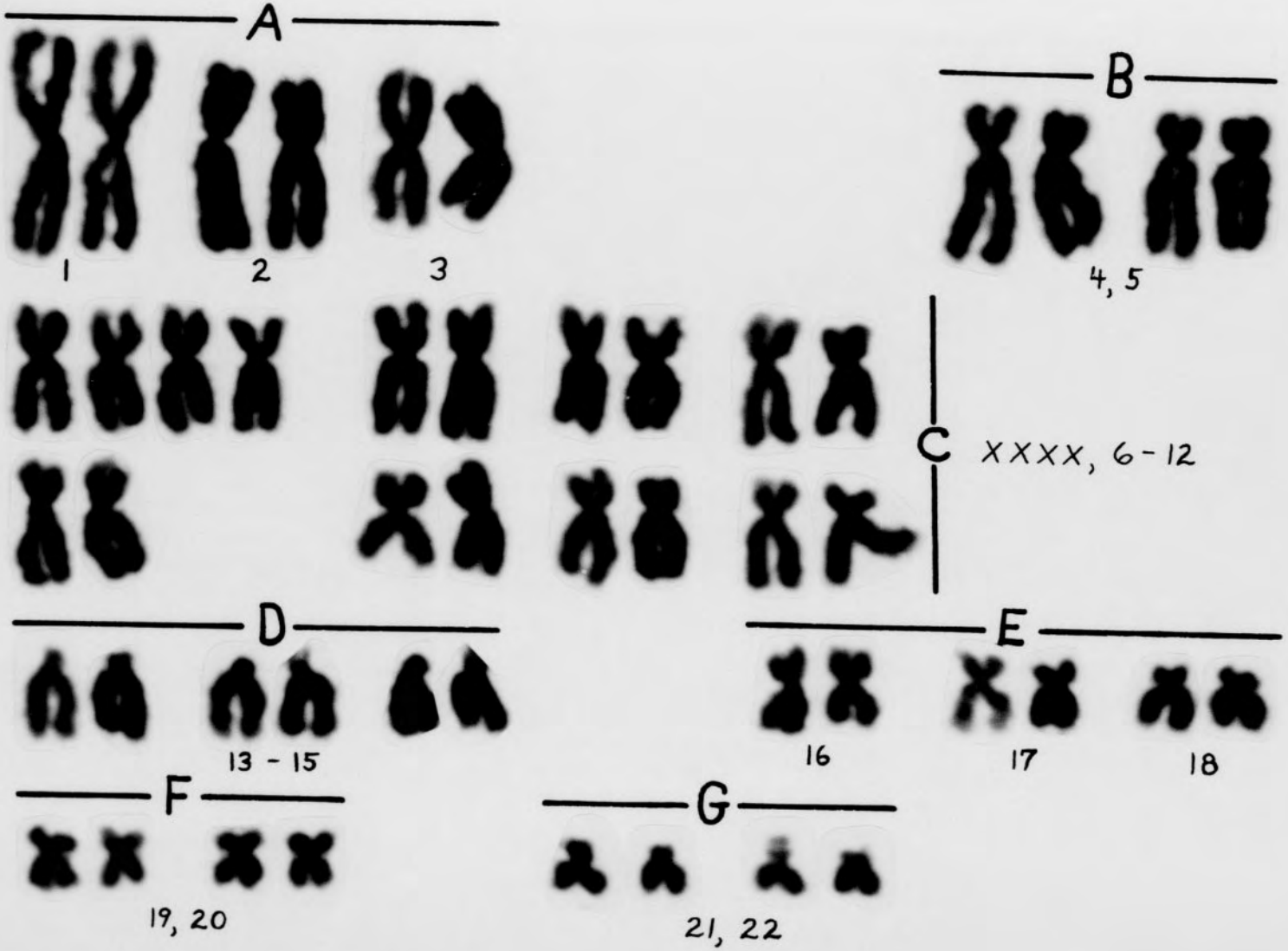


Karyotype	No. of chromosomes	Additional chromosome(s)	Missing chromosome(s)
No. 1	48	X,X	
No. 2	48	X,X	
No. 3	48	X,X	
No. 4	48	X,X	
No. 5	48	X,X	
No. 6	48	X,X	
No. 7	48	X,X	
No. 8	47	X,X	G
No. 9	47	X,X	G
No. 10	47	X,X	G
No. 11	47	X,X	G
No. 12	47	X,X	G
No. 13	47	X,X	G
No. 14	47	X,X	No. 3
No. 15	47	X,X	No. 3
No. 16	47	X,X	No. 3
No. 17	47	X,X	No. 3
No. 18	47	X,X	No. 3
No. 19	47	X,X	No. 16
No. 20	47	X,X	No. 16
No. 21	47	X,X	No. 16
No. 22	47	X,X	F
No. 23	47	X,X	F
No. 24	47	X,X	No. 1
No. 25	47	X,X	No. 1
No. 26	47	X,X	No. 17
No. 27	47	X,X	D
No. 28	47	X,X	B

Note the inconsistent loss of a chromosome in the non-modal cells. These findings were consistent with a diagnosis of tetrasomy X (Fig. 21, page 69).

Figure 21. Karyotype of case no. 30

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D
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Figure 22. Metaphase chromosome spread of karyotype, Fig. 21

e, Fig. 21

16. Electrophoresis of serum proteins (integrator counts)

Protein fraction	Case no. 30	XX mother	XY father	XY brother
Pre-albumin	1	2	3	3
Albumin	276	291	274	290
Post-albumin I	13	21	32	16
Post-albumin II	14	15		20
Post-albumin III	9	10	12	10
Transferrin	93	90	90	93
Unidentified I	9	10	6	9
Unidentified II	3	9	4	6
Unidentified III	8	10	10	15
Unidentified IV	10	10	10	20
Faster haptoglobin I	10	10	40	30
Faster haptoglobin II	40	40	60	20
Faster haptoglobin III	10	10	20	40
Gamma-globulin (7s)	70	70	50	50
Alpha-2 globulin	20	40	30	30
Slower haptoglobin I	10	10	10	10
Slower haptoglobin II	20	30	20	10
Beta-1 lipoprotein	60	50	70	50
Total counts	676	728	741	722

Clinical Report 8

1. Case no.: 34 2. Age: 5 weeks 3. Date of birth: 20 Feb. 1968

4. Race: White 5. Sex: phenotypic female

6. Date blood taken: 29 Mar. 1968

7. Age of mother at birth of propositus: 18

   Present state of health: living and well

8. Age of father at birth of propositus: N. R.

   Present state of health: N. R.

9. Siblings: none

10. Conceptual history: first pregnancy

11. History of pregnancy: uncomplicated

12. Labor: N. R.

    Delivery: N. R.

13. Birth weight: N. R. Length: N. R.

    Head circumference: N. R. Chest: N. R.

    Respiration: N. R.

14. Physical examination and clinical history

    Physical examination at birth revealed no neonatal problems except ambiguous genitalia. The infant was admitted to the Children's Home for adoption.

    The examining physician at the Children's Home requested sex-chromatin and chromosome studies because of the ambiguous genitalia.

15. Cytogenetic findings

Buccal smears for sex chromatin

Left cheek: sex-chromatin negative (none of 200 cells positive)

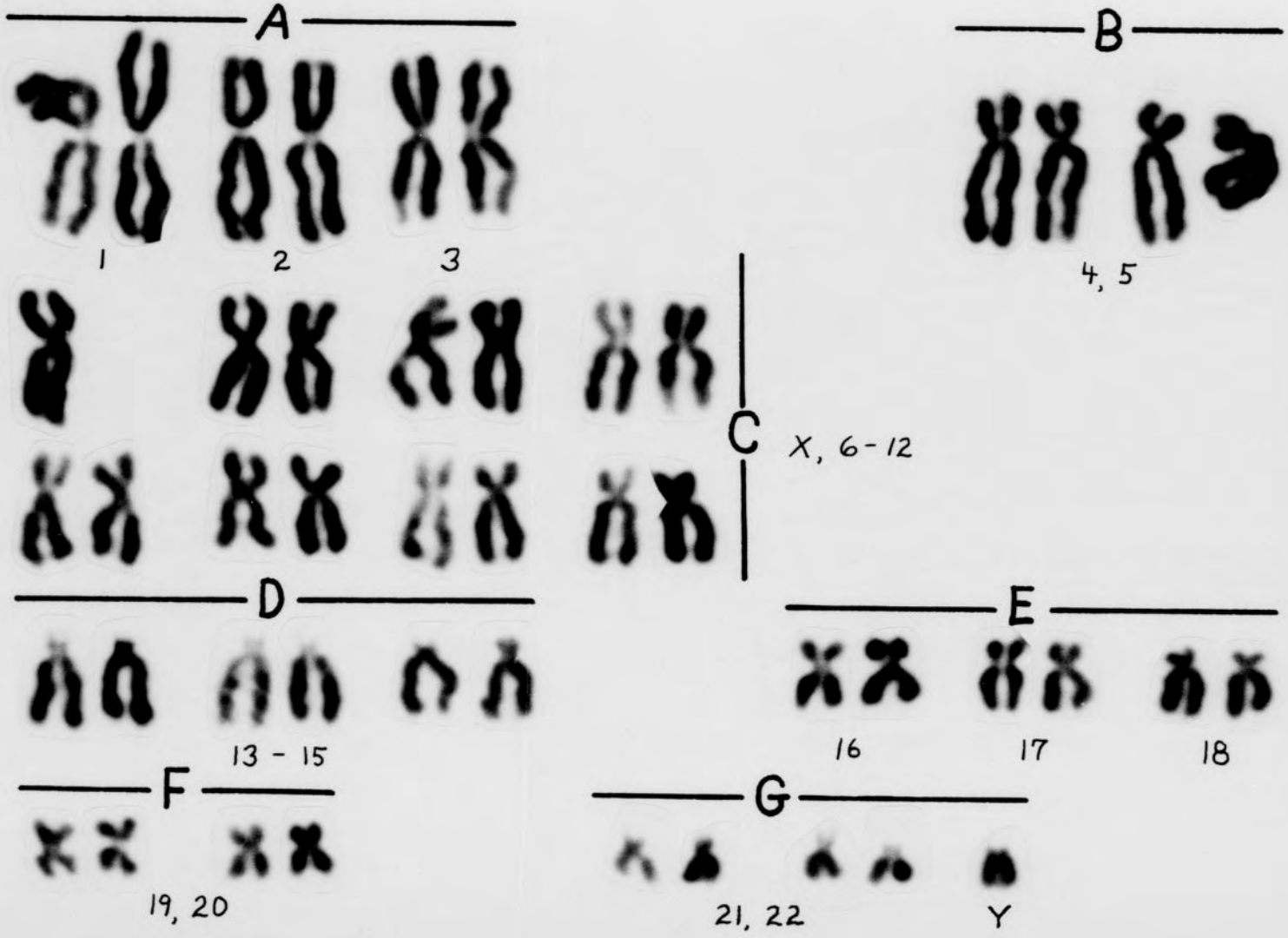
Right cheek: sex-chromatin negative (only one of 200 cells positive)

Chromosome counts	44	45	46	47	48	Polyploid	Total cells counted
No. of cells	4	3	91	1		1	100
% occurrence	4%	3%	91%	1%		1%	

A small acrocentric chromosome with the distinctive morphology of the Y chromosome was recognizable in 88 of the 100 cells counted.

Six karyotypes with 46 chromosomes had an XY sex chromosome complement (Fig. 23, page 76).

Figure 23. Karyotype of case no. 34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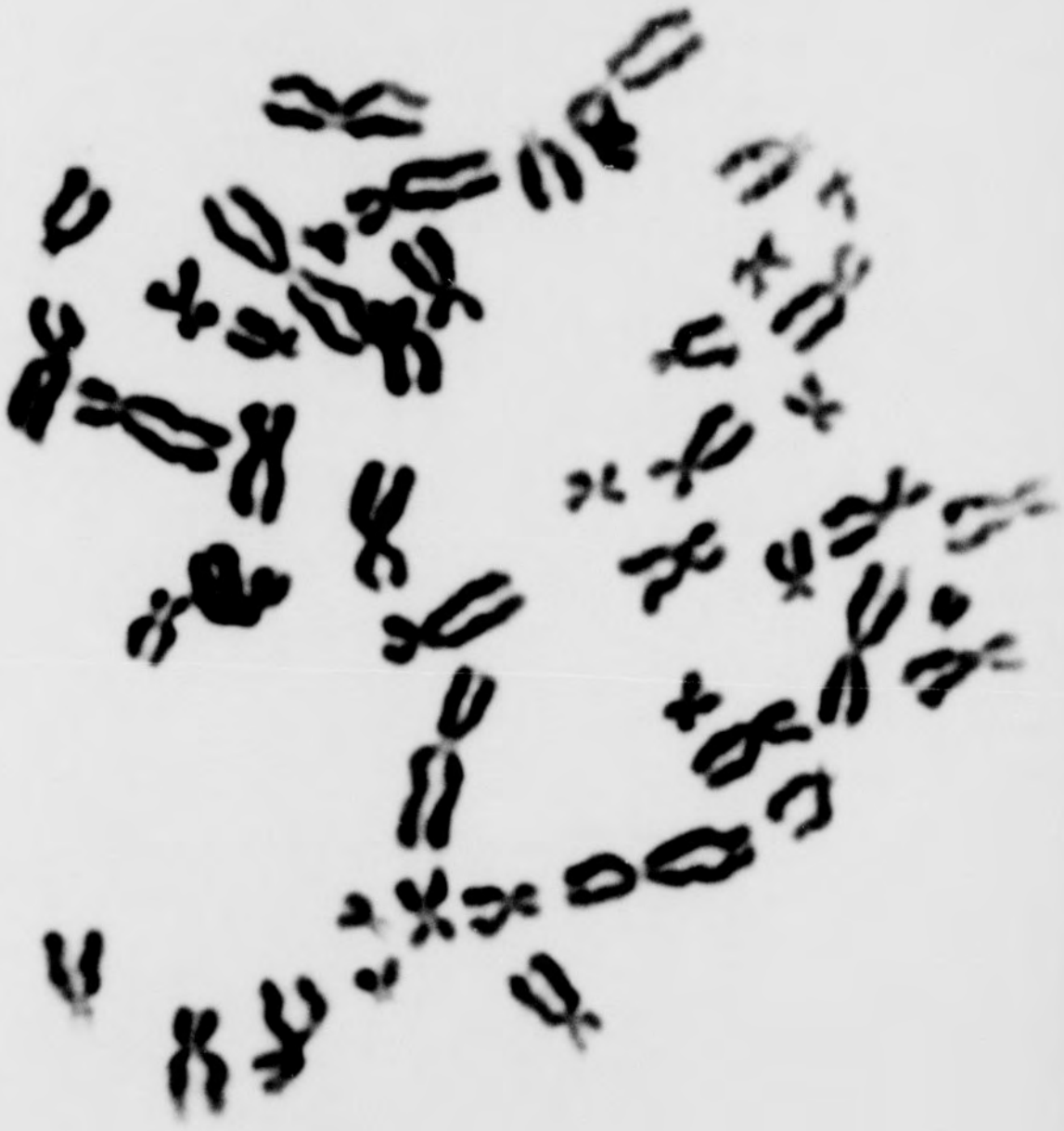
21, 22
Y

Figure 24. Metaphase chromosome spread of karyotype, Fig. 23



yotype, Fig. 23

16. Clinical follow-up

After cytogenetic studies were completed, the infant was admitted, at age 2 months, to the North Carolina Memorial Hospital, Chapel Hill, where it was clinically evaluated by the Division of Endocrinology, Department of Pediatrics. Physical examination revealed a completely bifid scrotum, or labia, with gonads palpable in each. Rectal examination revealed no palpable uterus. There was a small phallus with a ventral slit and no meatus on the phallus; the meatus was at the base of the phallus. No urogenital sinus or vagina could be seen. The infant voided through the meatus at the base of the phallus. Except for the genital ambiguity, no abnormalities were revealed by the physical examination and laboratory studies; 17-ketosteroid levels were within normal limits. It was the impression of the physicians that the infant was indeed a male. The question of feminizing testicular syndrome could not be completely ruled out, but it was felt that this syndrome would have resulted in more typical female genitalia. The endocrinologists felt that the infant probably could be reared as either a male or a female. Plastic surgeons were consulted and agreed that there was a reasonable chance that the infant could be made into a functional male, although the question of reproductive capability could not be resolved. It was therefore decided that attempts should be made to rear the infant as a male. A several-stage plastic surgical procedure was planned, the first stage of which was performed at age 4 months.

Clinical Report 9

1. Case no.: 43 2. Age: 4 months 3. Date of birth: 20 May 1968

4. Race: White 5. Sex: female 6. Date blood taken: 27 Sept. 1968

7. Age of mother at birth of propositus: N. R.

   Present state of health: N. R.; family history of heart disease and diabetes

8. Age of father at birth of propositus: N. R.

   Present state of health: N. R.

9. Siblings: N. R.

10. Conceptual history: N. R.

11. History of pregnancy: good health

12. Labor: N. R.

    Delivery: term

13. Birth weight: 7 lb. 3 oz. Length: 20½ inches

    Head circumference: N. R. Chest: N. R.

    Respiration: N. R.

14. Physical examination and clinical history

    Physical examination at birth revealed an unusual appearance. Rocker bottom feet and calcaneovalgus deformities were noted at the age of 1 week. The infant was placed in a boarding home by the Department of Welfare and was brought to the clinic at the age of 3 months because of "peculiar breathing." It was reported by the nurse that the infant appeared abnormal, with a small head and abnormal ears, that it was slow in development, and that it was experiencing difficulty in feeding because of poor suck and stopped-up nose. Physical examination revealed noisy respirations and a

very unusual appearance. The ears were simple and low-set and had abnormal folds. The neck was short, but there were no redundant folds. The eyes were very small, and there were slight bilateral epicanthal folds. Both hands showed a simian line. A skull film revealed that the head was normal in shape but abnormally small, comparing with the average size at birth. The physician recommended a series of plaster casts for the feet and physical therapy to stimulate developmental activities suitable to the infant's age.

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells		1	29			30
% occurrence		3.3%	96.7%			

Eight karyotypes with 46 chromosomes showed an apparently normal female chromosome complement (Fig. 25, page 83).

Figure 25. Karyotype of case no. 43

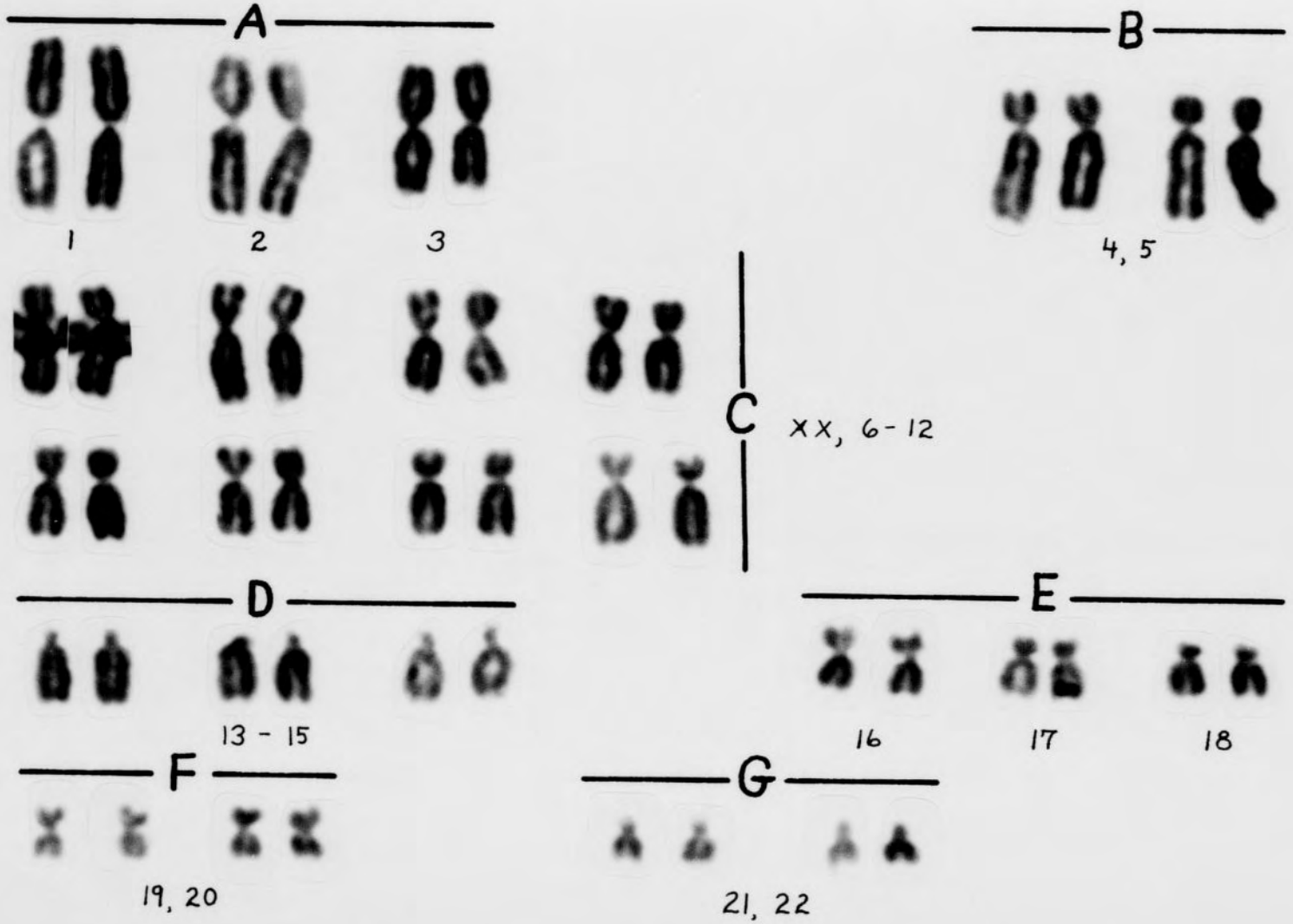

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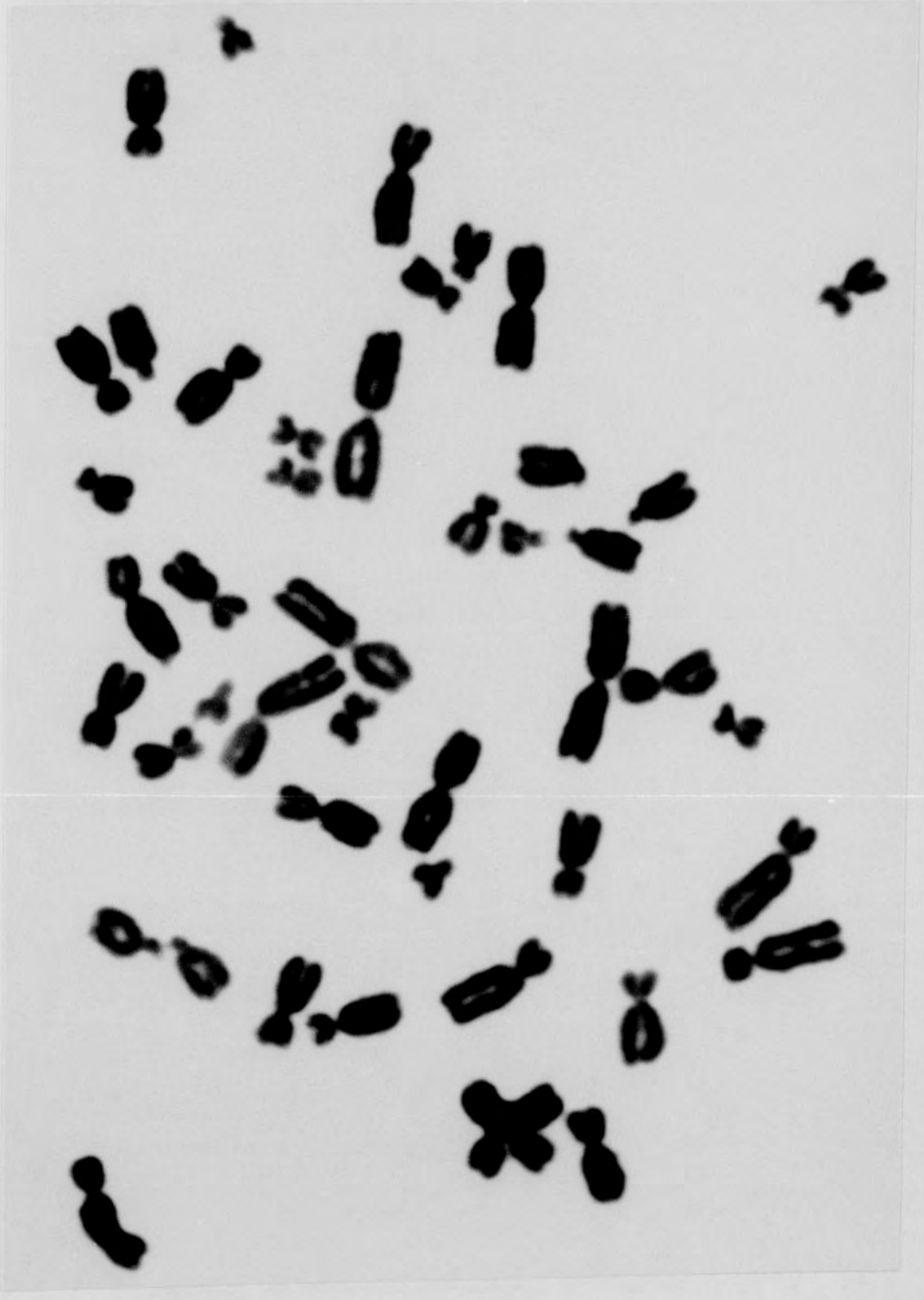
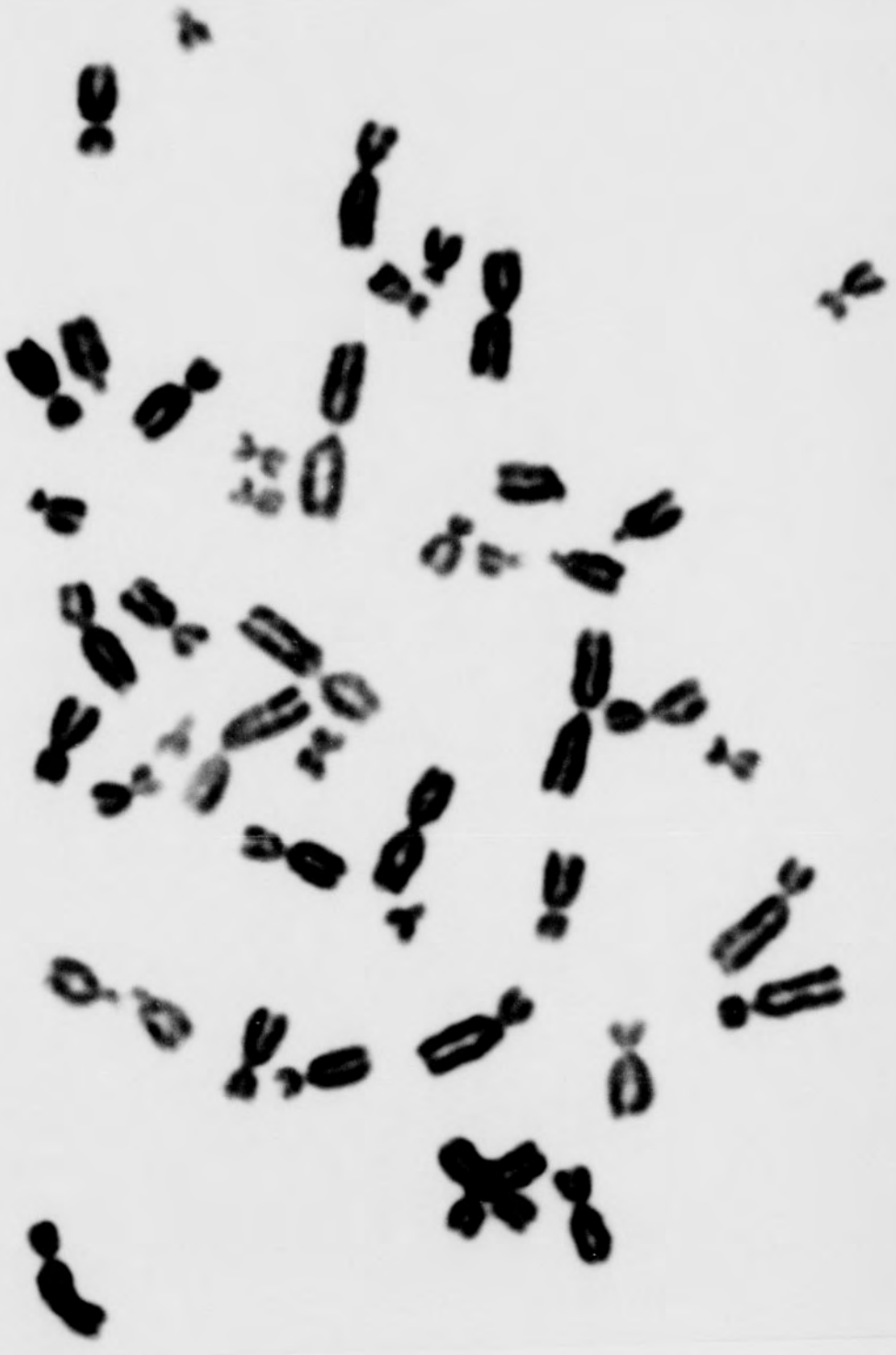
Figure 26. Metaphase chromosome spread of karyotype, Fig. 25



ryotype, Fig. 25



karyotype, Fig. 25

Clinical Report 10

1. Case no.: 47 2. Age: newborn 3. Date of birth: 11 Dec. 1968

4. Race: White 5. Sex: male 6. Date blood taken: 12 Dec. 1968
$12\frac{1}{2}$ hr. postmortem

7. Age of mother at birth of propositus: 28

Present state of health: has hives and is allergic to sulfa

8. Age of father at birth of propositus: N. R.

Present state of health: N. R.

9. Siblings: sister, age 5

10. Conceptual history: first pregnancy terminated at 7 months gestation with a live-born male weighing 3 lb. 13 oz. (expired after 36 hr.).

Second pregnancy resulted in living child, now age 5 (mother was in bed $4\frac{1}{2}$ months because of bleeding during pregnancy).

Third pregnancy resulted in abortion at 11 weeks gestation due to hemorrhage.

Fourth pregnancy resulted in premature delivery of the stillborn propositus.

11. History of pregnancy: spotting and cramping throughout this pregnancy

12. Labor: spontaneous, duration of $8\frac{1}{2}$ hr.

Delivery: 6 weeks premature, single footling breech

13. Birth weight: 2 lb. 10 oz. Length: 20 inches

Head circumference: $10\frac{1}{4}$ inches Chest: N. R.

Respiration: unresuscitable

14. Physical examination and autopsy report

Physical examination revealed a stillborn male infant with

macerated, wrinkled skin and multiple congenital anomalies. The placenta was small and fibrotic.

Gross examination at autopsy revealed low-set ears, questionable bilateral epicanthal folds, congenital anomalies of legs, feet, and hands, including bowed legs, everted feet, only three rudimentary toes on the right foot, dorsiflexion of the right hand, and inversion of the left hand. The nose was somewhat flat, the abdomen protuberant, and the liver palpable. A penis was present, but the scrotum was edematous and testicles were not identified in the scrotal sac. The head showed subcutaneous hemorrhage and edema.

Autopsy further revealed bilateral renal and ureteral agenesis, agenesis of rectum and anus with the descending colon ending in a blind pouch, small rudimentary bladder, and intra-abdominal small oval structures grossly consistent with testicles. The heart showed interventricular septal defect. Pulmonary hypoplasia and neonatal atelectasis were revealed. The brain appeared normal, both grossly and microscopically. Microscopic examination of the lungs further revealed parenchymal immaturity; the liver showed extramedullary hematopoiesis, and the umbilical cord had only two vessels. The diagnosis was given as Potter's syndrome.

15. Cytogenetic findings

Chromosome counts	44	45	46	47	48	Total cells counted
No. of cells	2	2	26			30
% occurrence	6.7%	6.7%	86.7%			

Seven karyotypes with 46 chromosomes showed an apparently normal male karyotype (Fig. 27, page 89).

Figure 27. Karyotype of case no. 47

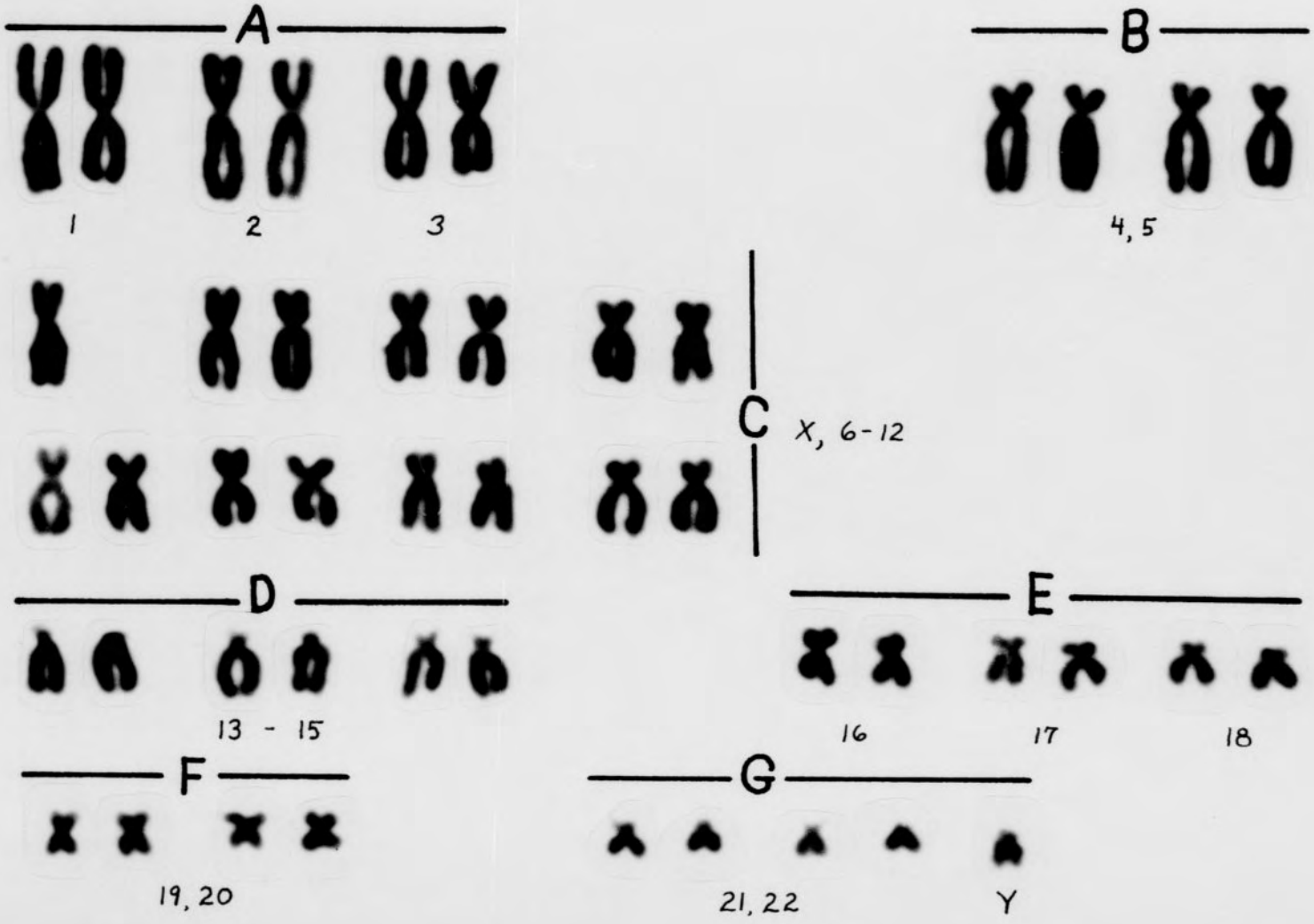

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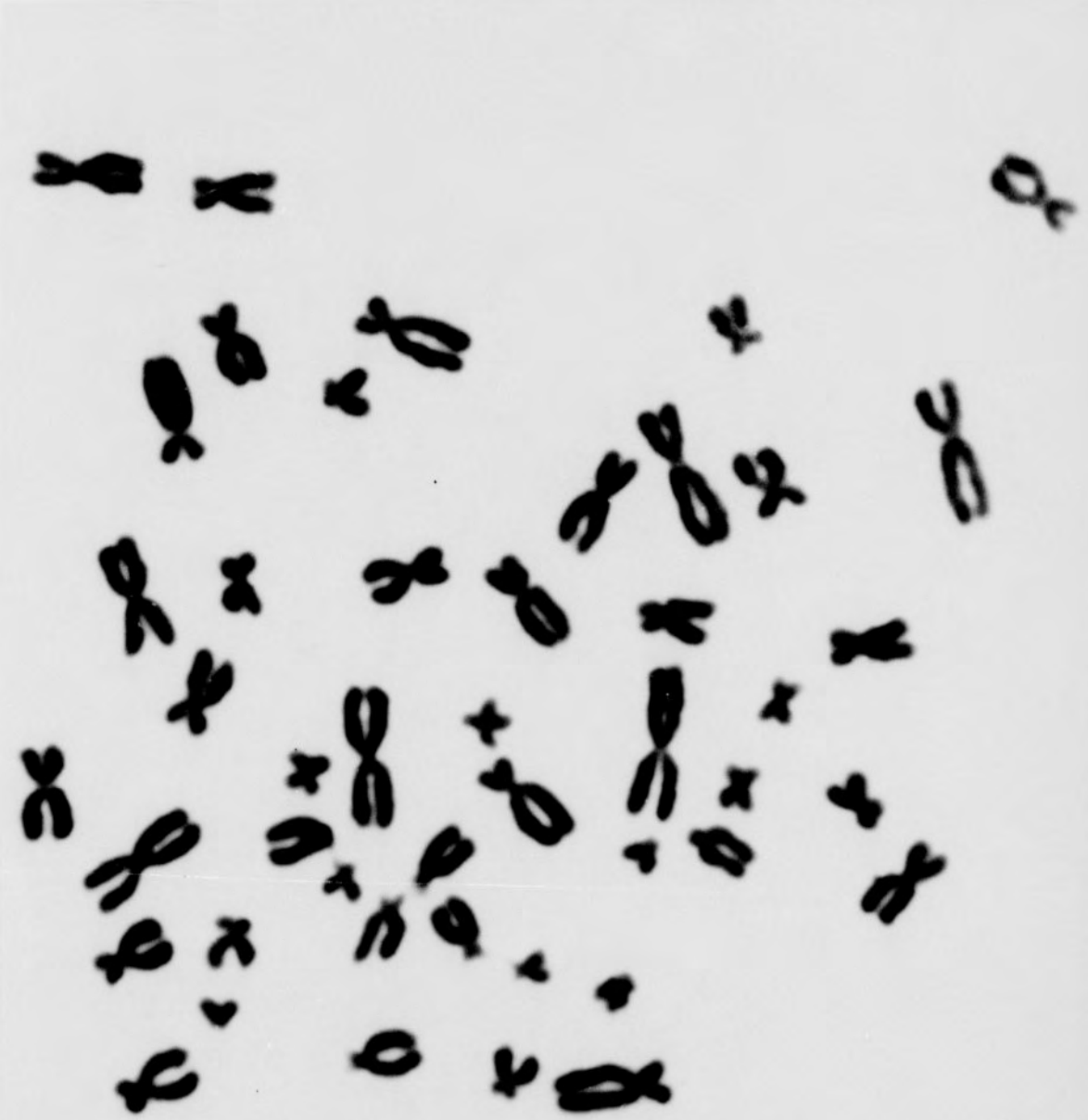
Figure 28. Metaphase chromosome spread of karyotype, Fig. 27



ype, Fig. 27

Maternal Age Studies

The only group with a sufficient number of cases for significant maternal age studies was the group with trisomy G (Down's syndrome). Distribution of the cases of Down's syndrome according to maternal age at birth is pictured in Figure 29 below. The mean maternal age was 30.5 years. The mean maternal age for all births is 28.5 years (Penrose, 1961).

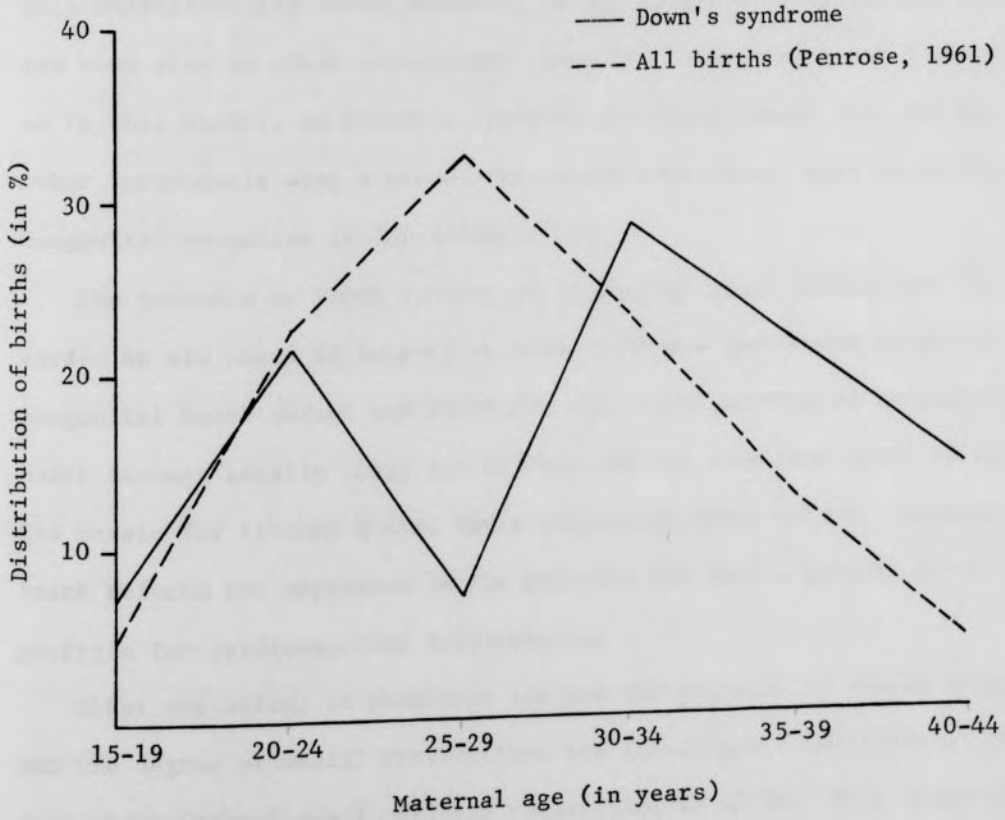


Figure 29. Distribution of the cases of Down's syndrome according to maternal age at birth

DISCUSSION AND REVIEW OF THE LITERATURE

Correlation of Phenotype and Karyotype

Trisomy G

Having analyzed the clinical information available, the writer noted one characteristic that was common to all 14 patients with trisomy G (Down's syndrome) -- the presence of epicanthal folds. The patient who was mosaic for trisomy G also had slight epicanthal folds. This characteristic alone, however, is not diagnostic; epicanthal folds are seen also in other chromosomal anomalies (e.g., tetrasomy X described in this study), in Potter's syndrome (Clinical Report 10), and in other individuals with a normal chromosome complement, with or without congenital anomalies (Clinical Report 9).

The presence or known absence of congenital heart disease was recorded in six cases of trisomy G; three patients were known to have a congenital heart defect and three did not. The question of congenital heart disease usually could not be resolved for some time after birth. The mosaic for trisomy G also had a congenital heart defect. Perhaps heart defects are expressed in the patients who have a genetic predisposition for cardiovascular difficulties.

Other variations in phenotype include the presence of simian creases and the degree of mental retardation; the intelligence quotients of the four older (school-aged) children ranged from 45 to 90. This range may simply reflect normal variation in intelligence due to heredity and cultural environment.

Congenital heart defects, simian creases, and mental retardation are also noted in other chromosomal anomalies and in individuals with normal karyotypes.

Langdon-Down (1866) first described this distinctive type of idiocy, referred to as "mongolian idiocy" because of the characteristic epicanthal folds. Other frequent findings are listed in Table 2, page 95. Lejeune, Turpin, and Gautier (1959) reported the chromosome number of nine affected children to be 47 rather than the normal diploid number of 46, having studied the metaphase chromosomes of fascia in culture. The extra chromosome was a small acrocentric one resembling one of those of the normal set (group G, originally thought to be a number 21, but now believed by some cytogeneticists to be a number 22). The incidence of Down's syndrome is one in 636-776 Caucasian births (Penrose, 1961).

Trisomy 18

Edwards et al. (1960) and Patau et al. (1960) simultaneously reported a new syndrome associated with trisomy of a group E chromosome, described as number 17 by Edwards, but now accepted as number 18. Frequent clinical findings are listed in Table 2, page 95. The prognosis for survival is grim; death usually occurs in early infancy. The immediate cause of death may be due to heart defects, aspiration, or respiratory failure. The incidence of this syndrome, estimated at between 0.23/1000 Caucasian births (Marden, Smith and McDonald, 1964) to as many as 1/500 births in a racially diverse population (Hecht et al., 1963), is reported to be three times greater in females than in males (Hecht et al., 1963); this disparity was attributed to greater lethality in male fetuses.

Table 2. Principal anomalies of selected syndromes

	Down's syndrome	Edwards' syndrome	Turner's syndrome	Klinefelter's syndrome	Potter's syndrome
Central nervous system	Mental retardation Hypotonicity	Mental retardation Hypertonicity	Mental retardation (sometimes)	Mental deficiency	
Cranium and facies	Epicanthal folds Brachycephaly Flat occiput Oblique palpebral fissures Hypertelorism Strabismus Nystagmus Cataract Furrowed tongue Irregular abnormal teeth Narrow high palate Short nose Abnormal earlobes	Prominent occiput Small mandible Low-set, malformed ears Small mouth	Epicanthal folds Low-set ears	Sparse facial hair	Epicanthal folds Small mandible Flattened bridge of nose Low-set, malformed ears Hypertelorism
Integumentary and musculoskeletal systems	Short stature Short neck	Mottled skin Retardation of osseous development Lanugo-like hair on back, extremities and forehead Hypoplastic nails	Cubitus valgus Short stature Webbed neck Low hair line Sparse pubic and axillary hair		
Extremities	Short, broad hands Short, incurved fifth finger Clinodactyly Gap between great and second toes	Flexion deformities of fingers with overlapping of index over third Dorsiflexion of short great toes Rocker bottom +/or equinovarus		Unusually long legs	Clubbing of hands and feet Other fixed and contracted joints
Thorax and pelvis	Frequent pulmonary infections	Short sternum Eventration of diaphragm Small pelvis Limited hip abduction	Broad shield-like chest with widely-spaced nipples and undeveloped breasts	Gynecomastia Sparse body hair	Pulmonary hypoplasia
Cardiovascular	Congenital heart lesions, especially septal and atrio-	Congenital heart defects, especially inter-	Coarctation of aorta		

pelvis	[illegible]	diaphragm Small pelvis Limited hip abduction	spaced nipples and undeveloped breasts	hair	
Cardiovascular	Congenital heart lesions, especially septal and atrioventricular canal defects Leukemia	Congenital heart defects, especially interventricular septal defects and patent ductus arteriosus	Coarctation of aorta		
Abdominal	Protuberant abdomen Duodenal atresia	Umbilical +/or inguinal hernia Meckel's diverticulum Heterotopic pancreatic tissue Horseshoe kidney Double ureter Funnel-shaped anus	Small uterus Ovaries represented by fibrous streaks Primary amenorrhea		Renal agenesis Absent vagina and uterus in females Imperforate anus
Genitalia			Infantile	Small testes with tubular hyalinization Small phallus Azoospermia	
Other		Altered gestation time Low birth weight Feeble cry Poor suck Single umbilical artery Small placenta Polyhydramnios	Congenital lymphedema Elevated urinary excretion of gonadotropins	Elevated urinary excretion of gonadotropins Decreased urinary 17-ketosteroids	Oligohydramnios Amnion nodosum Low birth weight Often breech presentation
Dermatoglyphics	Simian creases Ten ulnar loops on digits Distal axial triradii Radial loop on fourth finger Third interdigital loop Single distal crease on fifth finger Arch tibial or small loop distal in hallucal area of sole	Simian creases Single crease on fifth and other fingers Simple arches on all, or nearly all, fingers	Simian creases or partial simian creases Distal axial triradii Single crease on fifth finger		

Both cases of trisomy 18 described in this study were female, and both showed remarkably similar clinical abnormalities (phenotype), consistent with previously reported findings listed in Table 2. Both of these cases (case no. 1 and case no. 45) also showed abnormalities believed not to have been previously associated with this syndrome -- right facial weakness and asymmetry of the face.

45,X

Turner (1938) described a syndrome in females with short stature, webbed neck, and cubitus valgus; other common clinical findings in Turner's syndrome are listed in Table 2, page 95. These females were found to be sex chromatin negative (Polani, Hunter, and Lennox, 1954, and, separately, Wilkins, Grumbach, and Van Wyk, 1954). Ford et al. (1959) found a patient with Turner's syndrome to have a chromosome number of 45, with but a single sex chromosome, an X. The incidence of this syndrome is reported to be 0.4/1000 females (Maclean et al., 1964).

The physicians failed to note the presence or absence of many characteristic features of Turner's syndrome in the patient described in this study (case no. 29), but the congenital lymphedema and fibrous ovarian tissue are characteristic. The severity of the clinical symptoms indicates the extreme importance of a second sex chromosome in development, although it has been shown that X chromosomes above one are inactivated (at least to a great degree) in early embryonic life (Lyon, 1962).

The increased incidence of chromosome breaks and gaps (total 23.4%) was attributed to multiple X-rays (chest and I.V.P.) prior to the

cytogenetic study. The mean incidence in the general population, with comparable culture times, is reported to be 3.0% (Court Brown et al., 1966).

47,XXY

Klinefelter, Reifenstein, and Albright (1942) described a syndrome in males having small testes with tubular hyalinization; other clinical features are listed in Table 2, page 95. Affected males were shown by Bradbury, Bunge, and Boccabella (1956) and by Plunkett and Barr (1956) to be sex chromatin positive. Jacobs and Strong (1959) found Klinefelter's syndrome to have a chromosome number of 47 with two X chromosomes in addition to a Y sex chromosome. The incidence of all chromatin positive males is 1.96-3.10/1000 in Caucasians (Maclean et al., 1964, and Marden, Smith, and McDonald, 1964).

It appears that additional X chromosomal material is not so severe as the lack of a complete pair of sex chromosomes. The only phenotypic abnormality displayed by the patient described in this study (case no. 8) was a phallus that appeared small because of being embedded in a suprapelvic fat pad.

Tetrasomy X

Trisomy X in females was reported by Jacobs et al. (1959). Unlike other known trisomies, trisomy X does not present a constant phenotype; some individuals are phenotypically normal, but most have sexual abnormalities and/or mental deficiency. Sexual abnormalities, when present, range from mild menstrual disorders to amenorrhea and sterility. No characteristic dermatoglyphics are associated with this

trisomy. The incidence is 1.2/1000 births (Maclean et al., 1964). Females with more than three X chromosomes are extremely rare; two females with tetrasomy X were reported by Carr, Barr, and Plunkett (1961). Both were mentally retarded, with I. Q.'s of 30 and 50. Except for scant pubic hair and no axillary hair in the former, and moderate pubic hair and scant axillary hair in the latter, physical examinations and clinical histories were not remarkable. Menstrual histories and breast development (except for the presence of a small supernumerary nipple on the former patient) were normal. There is apparently a direct relationship between the number of extra X chromosomes and the degree of mental retardation.

The most striking finding in the female with tetrasomy X described in this study (case no. 30) was moderate mental retardation. Epicanthal folds were present, but they were also present in a normal (46,XY) brother and may therefore not be related to the chromosomal anomaly.

Note that a very low percentage of neutrophils were sex chromatin positive (0.4%, whereas the normal range has been found to be 1 to 10%). The four sex-chromatin positive cells each contained only a single drumstick, instead of the expected number of three (one less than the number of X chromosomes). Drumstick formation is dependent on the maturation process of the neutrophil (Mittwoch, 1963b). Extra X chromosomes have been found to inhibit the lobulation of neutrophils and the formation of drumsticks, so that the maximum number of drumsticks may rarely be reached; drumsticks may even be entirely absent (Mittwoch, 1963b, and Mittwoch, 1963a).

Note also the occurrence of Barr bodies in the buccal cells. In the normal female, 25% to 35% of the buccal cells are sex chromatin positive by the criteria used in this laboratory. The observed values 73.6%, 17.6%, 6.6%, and 2.2% for, respectively, zero, one, two, and three Barr bodies indicate that the probability of two or three Barr bodies occurring simultaneously (i.e., in the same cell) is the product of the individual probabilities ($0.264 \times 0.264 = 0.064$, and $0.264 \times 0.264 \times 0.264 = 0.017$).

This case of tetrasomy X, with accompanying mental retardation, lends further supportive evidence that all the X chromosomes have an active role, at least in embryonic development. According to Lyon (1962) all X chromosomes in a given cell except one are inactivated early in embryonic life and become tightly coiled, manifesting themselves as darkly staining sex chromatin bodies. Russell (1963) has demonstrated, however, that the region of inactivation of mammalian X chromosomes is limited. Perhaps a few, or many, genes on the X chromosome therefore remain active throughout life. Electrophoresis of serum proteins showed individual variation, in the haptoglobin fractions particularly, but it could not be concluded that genes controlling the synthesis of any of the serum proteins are located on the active region of the "inactivated" X chromosome(s); no dosage effect was demonstrated. The electrophoretic pattern of the female with tetrasomy X was remarkably similar to that of her normal 46,XX mother.

46,XY in a Phenotypic Female

Normally, if a Y chromosome is present, the phenotype is male. Male pseudohermaphrodites, or intersexes, however, fail to show all the characteristics of normal male genitalia; the degree of abnormality varies from slight anomaly to typical female external genitalia. Sex assignment is based upon the type of gonad present. The origin of intersexuality may be idiopathic, or it may be due to testicular feminization with a clearly genetic transmission (Stern, 1960). In the latter case, the external genitalia are typical female type, even with a small vagina; female secondary sex characteristics develop at puberty, and these individuals often marry males, but, of course, can bear no children. Male pseudohermaphroditism is suspected to arise from abnormal testicular hormones permitting or directing the development of embryonic and adult tissues into a female phenotype (Stern, 1960). In testicular feminization the testes secrete estrogens instead of androgens (Thompson and Thompson, 1966). It may prove important to determine whether the intersexuality is due to testicular feminization or not, in order to decide whether to rear the child as a male or as a female; the type of secondary sex characteristics anticipated at puberty will be a considerable factor in making the decision. Another factor to consider, however, in deciding to rear such a child as a female, is the possibility of a legal question arising, especially concerning marriage; there has not been a test case of this sort to date. Perhaps a major question to resolve in deciding on gender role is whether the individual could become either a functional male or a functional female; this apparently was the deciding factor for the patient described in this paper,

case no. 34. Early diagnosis and surgical repair in this case will make possible better development of the infant's biological potential, and he will be spared the trauma of having to change the gender role at puberty or adulthood. Hormone therapy will be given later in life if necessary.

46,XX in a Female with Multiple Anomalies

Case no. 43, although showing multiple congenital anomalies seen in the various chromosomal abnormalities, showed an apparently normal karyotype from cultured leukocytes. Certain characteristics of the patient were common to Edward's syndrome, e.g., low-set and malformed ears, rocker bottom feet, poor suck, and respiratory difficulty. However, other characteristics very common in Edward's syndrome were lacking in this patient, notably the characteristic crossing of the index fingers over the third fingers, prominent occiput, and small mandible. This patient also showed other clinical features seen in a variety of other chromosomal and non-chromosomal abnormalities, e.g., retardation, epicanthal folds, and simian creases. Although the pattern of anomalies does not fit exactly with a previously described syndrome, and the karyotype was apparently normal, these findings do not rule out a chromosomal abnormality too small to detect visibly. Nor does the apparent lack of mosaicism in leukocytes rule out mosaicism in other tissues.

46,XY in a Male with Potter's Syndrome

Potter (1946) described a syndrome of renal agenesis, with a striking tendency toward sex-linkage (17/20 cases were males); the incidence of this syndrome was found to be 0.3/1000 births. Frequent clinical findings in

Potter's syndrome are listed in Table 2, page 95. Many phenotypic abnormalities seen in Potter's syndrome are also seen in Edwards' syndrome, e.g., small mandible, low-set and malformed ears, fixed and contracted joints, respiratory and renal involvement (though these abnormalities differ in the two syndromes), and low birth weight. Again, however, the classic crossing of the index fingers over the third fingers, as seen in Edwards' syndrome, is absent in this syndrome.

Case no. 47, a typical case of Potter's syndrome in a stillborn male, showed an apparently normal male karyotype. This study confirms the findings of Passarge and Sutherland (1965), who found an apparently normal karyotype in three cases of Potter's syndrome. Again, these findings do not rule out a chromosomal abnormality not visibly detectable, or chromosome mosaicism in other tissues.

General Observations

One characteristic common to all the chromosomal anomalies in this study is mental deficiency of varying degrees. The mental deficiency and various developmental abnormalities are most likely due to an imbalance of the gene pool, resulting in interference with normal metabolism and cellular biochemistry. These metabolic defects may result in brain damage, altered growth rate, changes in inducing capacity and response to inducing agents (differentiation), and abnormal functioning of cells (Herskowitz, 1965). Mental deficiency and morphological abnormalities are not so pronounced in the sex chromosome anomalies, perhaps because of relatively few active genes as compared to the number contained in the autosomes.

Origin of Aneuploidy

One mechanism of aneuploidy (abnormal number of chromosomes) is meiotic nondisjunction. It is believed that the frequency of primary nondisjunction increases with advancing maternal age. Bodmer (1961) showed that aging in mice affects chromosome behavior in gametogenesis. In spermatogenesis there is continuous production of germ cells arising from spermatogonial divisions, but oögenesis occurs with the maturation of the relatively few ova present in the ovary at birth. The earliest stages of meiosis in the human female occur about the time of birth (Ohno et al., 1961a), then meiosis remains suspended following the diplonema stage until ovulation. It is believed that during this long stage of meiosis the chiasmata are progressively terminalized, so that chromosomes in old ova are entirely separated (Slizynski, 1960); it is therefore just a matter of chance whether the homologous chromosomes migrate to different poles of the dividing cell or to the same pole. Also, the satellited acrocentric chromosomes are associated at the satellites; satellite association was observed in 65.4% of the metaphases examined in this study. It is believed that these are the sites of nucleolar organization during interphase (Ohno et al., 1961b). Persistence of nucleoli during cell division can interfere with pairing of chromosomes in meiosis, and the unpaired chromosomes are subject to nondisjunction. It is thought that the long dormant stage of oögenesis causes some change in the nucleolus so that it becomes more resistant to breaking down when meiosis resumes (Ohno et al., 1961b). The maternal age effect is pronounced in the incidence of trisomy G, and is also noted to a lesser degree in the incidences of the other autosomal

trisomies, Klinefelter's syndrome, and multiple X karyotypes (Milham and Gittelsohn, 1965). The distribution of the 14 cases of trisomy G in this study according to maternal age at birth (Fig. 29, page 92) indicated a correlation between the incidence of trisomy G and advanced maternal age.

Another mechanism of aneuploidy is mitotic nondisjunction in the zygote. Mitotic nondisjunction may, on the other hand, serve to restore the normal karyotype (euploidy) in a zygote that was originally trisomic. In either event, mosaicism is frequently the result; the affected individual, however, may show only one cell type or the other in the tissues selected for cytogenetic study. In case no. 38, the mosaic for trisomy G, the mitotic nondisjunction resulting in mosaicism may have produced the aneuploid cell line in an originally normal zygote, or it may have produced a normal cell line in a zygote originally trisomic for the G chromosome. The family history indicates a possible genetic predisposition for congenital defects, possibly attributable to chromosomal abnormalities. Evidence for genes increasing the incidence of chromosomal aberrations is slight; it is based upon the observation of increased consanguinity in maternal grandparents of subjects with mongolism, and upon studies of families with more than one type of chromosomal abnormality affecting its members, or even the same individual (Hecht et al., 1964). Chromosomal aberrations such as inversions, resulting in nondisjunction due to the failure of chromosome pairing, may also be inherited and may account for a genetic predisposition for certain congenital defects (Stewart, 1960). It is important in genetic counseling to determine the existence and the extent of mosaicism, i.e., the percent of each cell

type in a given tissue and the different tissues affected. The prognosis may be more favorable if 50% of the cells have a normal karyotype.

Nondisjunction is believed to be associated also with radiation, hormonal imbalance, certain viruses, and autoimmunity. Radiation is known to produce chromosome breaks and structural rearrangements (Muller, 1947), which may result in nondisjunction due to failure of pairing. Carr (1967) has suggested that hormonal imbalance may be the cause of increased triploidy, based on his findings of triploidy and 45,X in abortuses of women who had been taking oral contraceptives at the time of conception. See Fig. 30, page 108, for endoreduplication of chromosomes in a female, age 24, with primary amenorrhea presumed due to hormonal imbalance. Fialkow (1966) found autoimmunity to be associated with Down's syndrome and with sex chromosome anomalies. Robinson and Puck (1965) found a much greater incidence of trisomy G and extra X chromosomes following a rubella epidemic; Stoller and Collmann (1966) found a highly significant correlation between the incidence of infectious hepatitis and Down's syndrome nine months later for mothers aged 35 years and over, but not for younger mothers.

The many factors involved in the incidence of chromosomal abnormalities make mandatory the taking of a more complete history by the physician. The presence or absence of consanguinity, congenital defects in the kinship, hormonal imbalance, autoimmunity, exposure to X-rays and drugs, and viral diseases should be noted on the expectant mother's chart. The history of the pregnancy should also include abnormalities such as delayed quickening or relative inactivity of the fetus. Any abnormality at delivery, e.g., of the placenta, amniotic fluid, or the presentation,

should be noted. Breech presentation has been associated with abnormalities of the infant's hips or legs, as seen in Potter's syndrome (Passarge and Sutherland, 1965) and in congenital dislocation of the hip (Carter, 1965). Cases of Edwards' syndrome, with accompanying limited hip abduction, should be reviewed for a possible increased incidence of breech presentation.

Figure 30. Endoreduplication of chromosomes

SUMMARY

Cytogenetic investigation of 46 selected children with birth defects was carried out by culture of peripheral leukocytes to determine (1) whether a chromosomal anomaly could be demonstrated, (2) the correlation between karyotype and phenotype, and (3) dosage effect in cases of aneuploidy by electrophoresis of serum proteins.

This study revealed 20 abnormal chromosome complements and one XY sex chromosome complement in a phenotypic female. There was some individual variation in phenotype among those affected by a given chromosomal anomaly, probably due to differences in genetic background and in maternal host environment. Abnormalities in development common to two or more different chromosomal anomalies were also noted, mental deficiency being common to all. However, there was a relatively consistent and distinct over-all pattern of development (phenotype) with each chromosomal abnormality, which should allow a specific clinical diagnosis.

Electrophoretic studies of serum proteins in a female with four X chromosomes, normal XX females, and normal XY males failed to show a dosage effect, indicating that genes for synthesis of serum proteins are not located on the active region(s) of the "inactivated" X chromosome(s). It is hoped that further biochemical studies may be done on this patient and on patients with autosomal trisomies and chromosome deletions, in an effort to map genes on human chromosomes and to indicate appropriate clinical treatment for these patients.

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APPENDIX

Leukocyte Culture Medium

Medium 199 with $NaHCO_3$ (Microbiological Associates, Inc.)	800 ml
Fetal bovine serum (Microbiological Associates, Inc.)	200 ml
Bacto-Phytohemagglutinin - M, aqueous solution (Difco)	20 ml
Heparin sodium, 1000 units/ml (Nutritional Biochemicals Corporation)	20 ml
Penicillin - Streptomycin, 5,000 units each/ml (Microbiological Associates, Inc.)	20 ml

The culture medium was dispensed in 5-ml quantities into Kimble "Opticlear" vials, size 4; the vials were then capped with plastic caps previously rinsed in 70% alcohol. The vials of culture medium were stored in the freezer at -15°C and thawed immediately before use.