Viral Infections and Chromosome Aberrations

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INTRODUCTION

Related to the progress of clinical, fundamental studies of cytogenetics during the last ten years, there has been increasing interest in the problem of human chromosome aberrations. It is necessary that one should pay extensive attention to the relation between the infection of teratogenic, oncogenic viruses and chromosome aberrations in human beings. Until now, many chromosome studies, in vitro and in vivo, have been reported in virus-infected cell systems. Now it is clear that a variety of types of breakages are induced with viruses in many cell systems, but its mechanism is still unknown.

This report presents chromosome studies in peripheral blood leukocytes of man in various viral infections; 197 cases of rubella, mumps, varicella, measles, viral meningo-encephalitis, viral pneumonia, infectious hepatitis, infectious mononucleosis, Guillain-Barré's syndrome and herpes zoster, and in peripheral blood leukocytes of African green monkeys infected with rubella virus (in vivo). Moreover chromosome studies in many cultured cell systems, BHK (baby hamster kidney), RE (rabbit embryo), GMK (green monkey kidney) and HEK (human embryonic kidney) cells, infected with rubella virus and adenoviruses are attempted.

In these observations it became rather clear that chromosome aberrations, various types of breakages are relatively higher in virus-infected cell systems.

Contents;

1) Chromosome studies of cultured peripheral blood leukocytes on viral infections.
2) Chromosome studies of cultured peripheral blood leukocytes on African green monkeys infected with rubella virus (in vivo).
3) Chromosome studies of various cultured cell systems infected with rubella virus and adenoviruses.
   (1) Chromosome studies of cultured BHK, RE and GMK cells infected with rubella virus.
   (2) Chromosome studies of cultured BHK and HEK cells infected with adenoviruses.

1) Chromosome studies of cultured peripheral blood leukocytes on viral infections.

Materials and Methods

The subjects are consisted of 197 cases of virus infected patients; 64 cases of rubella prevailed largely in Ube city in 1966 and others prevailed ramdomly; 40 cases of mumps, 20 cases of varicella, 12 cases of measles, 46 cases of viral meningo-encephalitis, 7 cases of viral pneumonia, 3 cases of infectious hepatitis, 2 cases each of infectious mononucleosis and Guillain-Barré's syndrome and 1 case of herpes zoster. Of these patients, 117 cases were male and 80 cases were female, ranged from 0 to 15 years in age.

Each patient was diagnosed with typical clinical features and general examinations. Partially in rubella patients virological studies were performed for diagnosis (Nohara, K., 1968). The days of sampling were determined by exanthem in rubella, varicella, measles, infectious mononucleosis and herpes zoster, subauricular swelling in mumps, fever and cough in viral pneumonia, meningeal signs in meningo-encephalitis and jaundice in infectious hepatitis.

Chromosome studies were carried out on peripheral blood leukocytes, taken from the acute phase to one month later, using a slight modification of the technique of Moorhead, et al. (1960). (Konishi, S., et al., 1967).

Methods of chromosome preparations in cultured human peripheral blood leukocytes;

a) Draw 5-10 ml. venous blood into a sterile syringe with heparin (1 mg./ml.) 1 ml. Pour into a sterile tube and allow to stand for 30–60 minutes at room temperature.

b) Collect plasma 1.5-2 ml. and mix with LYG (lactalbumin-yeast-glutamine-Hanks) medium 6 ml. Divide into two TD-15 Earle flasks.

c) Add PHA (Phytohemagglutinin M) 0.05 ml. on each flask. Incubate for 60-72 hours at 37° C.
(necessary of sterile procedure in a) to b) process)

d) Add colchicine (50 mg./ml.) 0.05 ml. to each flask. Incubate for 2-3 hours again.

e) Strip cells from flasks by shaking. Remove into a centrifuge tube and centrifuge at 800 rpm. for 5-7 minutes.

f) Add 0.5 % sodium citrate solution to the sediment. Mix and allow to stand for 20 minutes in a 37° C incubator. Centrifuge as before.

g) Add Carnoy solution to the sediment. Mix and stand for 20 minutes at room temperature. Repeat this method 2 times.

h) Add suitable Carnoy solution to the sediment. Drop 2 pipets of the solution to purified slide glass in wet with 70 % alchohol. Dry immediately with

hair drier.

i) Stain with 20-hold Giemsa solution. Dehydration, dry and clear in xylol. Mount with bioleit.

On each case 50 to 100 well-spread metaphase plates were counted for numeral and structural chromosome aberrations.

As the controls, 15 somatically healthy children (control 1) and 10 hospitalized children of the same age suffering from asthma and nephrosis (control 2), were examined for frequency of chromosome breakages in cultured peripheral blood leukocytes by the same methods.

Chromosome breakages such as breaks, fragments, interchanges, dicentrics and endoreduplications were decided according to the definition of Bloom, et al. (1966).

"Chromatid exchanges are abnormal chromosomal groupings apparently arising as a result of chromatid breaks in 2 chromosomes, followed by a reunion of genetic material."

"In endoreduplication, chromosomal doubling occurs without complete separation of the partners of each pair, so that at metaphase the cell is composed of 46 four-stranded structures."

"The chromatid breaks that were seen involved either one or both chromatids of a chromosome. In some cases an outward rotation or angulation of the distal fragments occured ; in others, chromosomal material distal to a break was missing."

"Chromatid gaps consisted of achromatic segments without displacement of the distal fragments."

Besides this, the author defined as follows ; breaks or fragments less than 3/metaphase as one positive, 4-6/metaphase as two positive and more than 7/metaphase (diffusely damaged fragmentation involved) as three positive.

Results

As seen in Table 1, 73 of cases (37.1 %) of the virus infected patients had a relatively high frequency of chromosome breakages beyond 8.0 % of breakages in upper limits of the controls. Of 64 cases of rubella patients 42.1 % showed a high frequency of abnormal metaphase plates with an average of 8.0 %. And, concerning with 40 mumps, 20 varicella, 12 measles, 46 meningo-encephalitis, 7 viral pneumonia, 3 infectious hepatitis, 2 infectious mononucleosis, 2 Guillain-Barré's syndrome and 1 herpes zoster-patients, each of them showed 17.5, 50.0, 50.0, 14.2, 39.1, 33.3, 100, 0, 100 % respectively showing more than 8 % of abnormal metaphase plates with an average of 4.8, 8.5, 8.2, 5.9, 6.5, 18.3, 21.5, 7.0, 12.0 %. The highest chromosome aberrations of 24.0, 14.0, 23.0, 13.0, 16.0, 9.0, 32.5, 33.0, 7.0, 12.0 % were shown during the first week of the acute stage (Table 2–8, Fig. 1–7). For the total cases 7.4 % of abnormal metaphase plates (1167 of 155703) were moderately different comparing with 4.1 % of control 1 and 3.2 % of control 2 (Table 9).

Table 1. Chromosome studies in peripheral blood leukocytes on cases of various viral diseases

viral diseases	number of cases	aberrations, more than 8 % (%)	total (%)
rubella	64	27 (42.1)	8.0
mumps	40	7 (17.5)	4.8
varicella	20	10 (50·0)	8.5
measles	12	6 (50.0)	8.2
viral pneumonia	7	1 (14.2)	5.9
encephalitis and meningitis			
mumps-	33	12	
aseptic-	11	5	
rubella-	1	1	
measles-	1	0 (39.1)	6.5
infectious hepatitis	3	1 (33.3)	18.3
infectious mononucleosis	2	2 (100)	21.5
Guillain-Barré's syndrome	2	0 (0)	7.0
herpes zoster	1	1 (100)	12.0
total	197	73 (37.1)	7.4

Table 2. Rubella

[a]	[b]	[c]	[d]								
1	8, M	1	4.0	23	13, F	2	9.0	43	13, M	7	4.0
2	13, M		15.0	24	14, F		7.4	44	13, M		5.0
3	5, F		2.0	25	7, M	3	4.0	45	14, M		11.0
4	7, F		2.9	26	8, M		14.0	46	14, M		11.0
5	7, F		0	27	12, M		15.0	47	15, M		7.0
6	8, F		22.0	28	12, M		13.0	48	15, M		4.0
7	8, M	2	20.0	29	13, M		6.0	49	11, F		7.7
8	8, M		11.0	30	11, F		8.0	50	13, F		11.0
9	9, M		6.0	31	4, M	4	4.0	51	13, F		4.0
10	9, M		6.0	32	9, M		10.0	52	15, F		22.7
11	9, M		5.0	33	11, F		7.0	53	15, F		8.3
12	9, M		4.0					54	15, F		6.0
13	9, M		2.0	34	5, M	5	3.0	55	15, F		0
14	12, M		24.0	35	4, F	6	0	56	12, M	8	14.3
15	13, M		9.8					57	8, F		4.4
16	8, F		9.0	36	6, M	7	3.3	58	12, M	14	3.0
17	8, F		3.0	37	8, M		10.0	59	13, M	28	13.0
18	9, F		21.0	38	9, M		7.0	60	13, M		0
19	9, F		16.0	39	10, M		10.0	61	14, M		9.0
20	9, F		4.0	40	11, M		17.0	62	11, F		2.0
21	9, F		1.0	41	11, M		5.0	63	12, F		0
22	12, F		5.0	42	13, M		8.0	64	15, F		6.1

Table 3. Mumps

1	5, M	1	0	22	12, M	5	8.0
2	5, F		4.0	23	8, F		5.0
3	9, F		3.3	24	8, F	7	7.0
4	6, M	2	11.0	25	11, F		7.0
5	12, M		3.0	26	10, M	8	2.0
6	14, M		4.0	27	8, F		1.0
7	4, F		12.5	28	9, F		7.0
8	4, F		5.0	29	12, F		3.0
9	5, F		2.0	30	12, M	9	11.0
10	5, M	3	14.0	31	10, M	10	6.0
11	5, M		0	32	10, M		4.0
12	6, M		8.0	33	14, M		2.0
13	10, M		5.0	34	10, F	11	2.0
14	7, F		0	35	11, F		6.7
15	9, F		2.0	36	13, F		9.0
16	11, F		4.0	37	14, M	21	2.0
17	11, F		3.0	38	7, F		4.0
18	11, F		3.0	39	11, F	28	2.0
19	6, M	4	5.0	40	13, F		6.0
20	13, F		5.0				
21	13, F		3.3				

Table 4. Varicella

1	4, M	2	2.0
2	6, M		0
3	5, M	3	0
4	7, M		16.0
5	2, F		8.0
6	7, F		7.0
7	12, F		8.0
8	12, F		6.0
9	3, M	4	23.0
10	4, M		11.0
11	4, M		6.0
12	5, M		2.0
13	6, M		4.0
14	3, F		13.0
15	6, M	5	5.0
16	5, F		10.0
17	4, M	7	6.0
18	5, M		21.0
19	6, M		8.0
20	10, M		13.0

Table 5. Measles

1	5, M	3	17.0
2	7, M		1.0
3	4, F		0
4	0, M	4	6.0
5	5, M	5	10.0
6	6, F		13.0
7	6, F	6	3.0
8	2, M	7	10.0
9	4, F		10.0
10	3, M	21	10.0
11	6, M		2.0
12	5, M	28	0

a : case number
b : age and sex
c : days of sampling
d : % of abnormal cells

Table 6. Meningitis and encephalitis

mumps meningitis			
1	4, M	2	12.0
2	5, M		14.3
3	6, M	3	10.0
4	11, M		5.0
5	2, F		3.0
6	4, F		8.0
7	5, M	4	5.0
8	5, M		0
9	8, M		10.0
10	6, F		13.0
11	3, M	5	6.0
12	4, M		8.0
13	4, M		6.0
14	5, M	6	6.0
15	3, M	7	7.0
16	4, M		6.0
17	5, M		7.0
18	5, M		4.0
19	2, F		8.0
20	5, F		3.0
21	6, F		16.0
22	6, F		3.0
23	8, F		8.0
24	4, M	8	8.0
25	3, M	12	6.0
26	4, M		7.0
27	7, M		5.0
28	6, F	14	0
29	6, M	15	4.0
30	8, M	17	6.0
31	3, M	19	3.0
32	4, M		5.0
33	6, F	21	13.0
aseptic meningitis			
1	3, M	2	8.0
2	6, M	3	7.0
3	7, F		9.0
4	4, M	5	8.0
5	4, M		2.0
6	4, M	6	4.0
7	5, M	7	3.0
8	8, M		5.0
9	7, F		10.0
10	10, M	9	8.0
11	3, F		4.0
rubella encephalitis			
1	14, F	3	10.0
measles encephalitis			
1	6, M	90	0

Table 7. Viral pneumonia

1	6, M	7	0
2	7, M	11	6.0
3	5, M	14	7.0
4	5, F		9.0
5	8, F	18	3.0
6	5, M	28	2.0
7	7, F		6.0

Table 8. Other viral diseases

infectious hepatitis			
1	7, M	21	17.0
2	11, M		32.5
3	6, M	28	0
infectious mononucleosis			
1	5, M	14	10.0
2	3, F	28	33.0
Guillain-Barré's syndrome			
1	5, F	21	7.0
2	14, F	28	7.0
herpes zoster			
1	11, M	4	12.0

Table 9. Incidence of chromosome breakages in peripheral blood leukocytes of control cases

control 1. (healthy children)	
case no.	(%) of abnormal cells
1	7.0
2	7.0
3	3.0
4	3.0
5	6.0
6	7.0
7	4.0
8	6.0
9	1.0
10	2.0
11	7.0
12	0
13	0
14	4.0
15	4.0
average	4.1 (%)

control 2. (children used predonisolone)	
case no.	(%) of abnormal cells
1 3, M nephrosis	1.0
2 6, M	5.0
3 6, M	2.0
4 8, M	4.0
5 15, M	6.0
6 16, M	7.0
7 1, F	4.0
8 7, M asthma	0
9 12, M	3.0
10 12, F	0
average	3.2 (%)

Fig. 1. Distribution of breakages on rubella

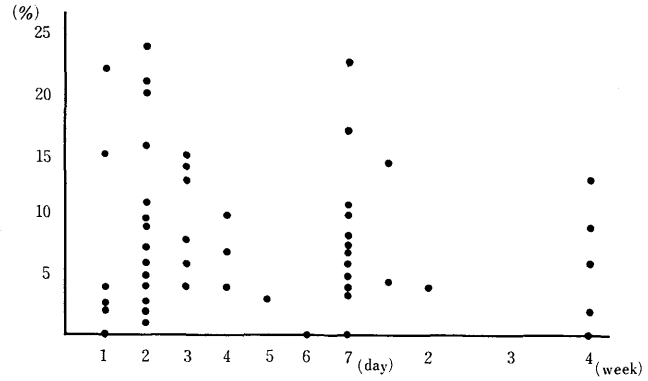


Fig. 2. Distribution of breakages on mumps

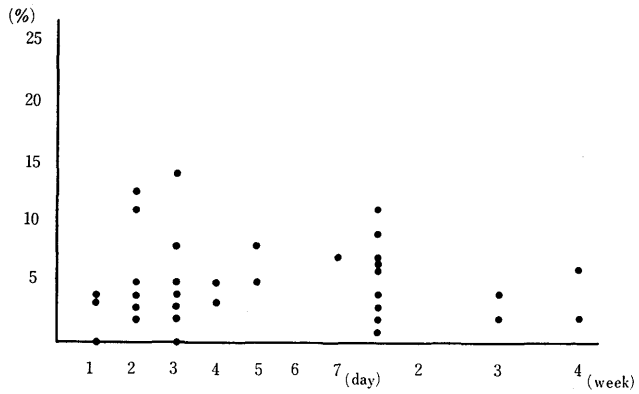


Fig. 3. Distribution of breakages on varicella

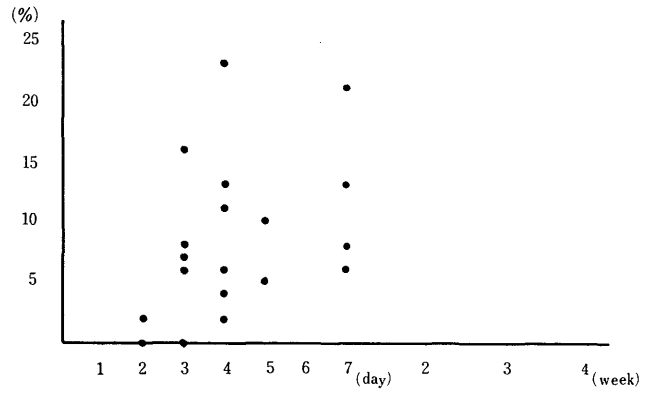


Fig. 4. Distribution of breakages on measles

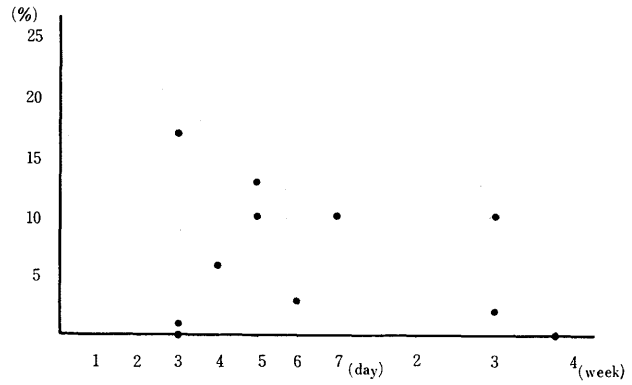


Fig. 5. Distribution of breakages on meningitis and encephalitis

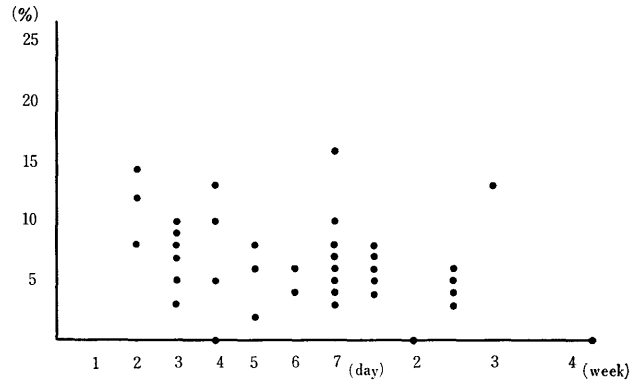


Fig. 6. Distribution of breakages on viral pneumonia

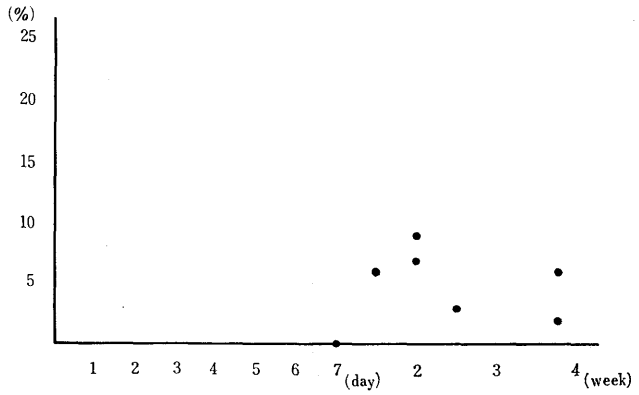
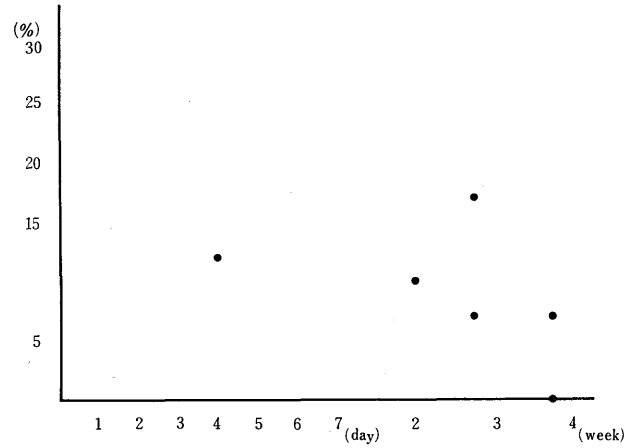


Fig. 7. Distribution of breakages on other viral diseases



The most common type of aberrations in virus infected patients consisted of chromatid or isochromatid breaks and fragments (99.1 %) shown as one to three positive. A few interchanges, dicentrics and endoreduplication (0.9 %) were noted in contrast to 0 % in controls (Table 10).

Table 10. Breakages in peripheral blood leukocytes on virus infected patients (%)

	break or fragment (+) (++) (+++)	interchange	dicentrics	endoreduplication
subjects (%)	96.7 2.1 0.3	0.4	0.4	0.1
controls (%)	100			

The distribution of breakages in the different chromosome groups was proportional to the chromosome length with more than 90 % in groups A, B, C, D and more than 70 % in the long arms of the chromosomes. This is almost similar to the control cases (Table 11, 12).

Except for 2 cases of 21-trisomy and 1 case of YY-syndrome, all other cases of virus infected patients had a modal number of 46 chromosomes and normal karyotypes.

2) Chromosome studies of cultured peripheral blood leukocytes on African green monkeys infected with rubella virus (in vivo).

Materials and Methods

Five cases of healthy African green monkeys were infected with rubella virus. Chromosome aberrations were observed for the period of 15 days after infection, using the method of whole blood cultures according to the above-mentioned techniques from human peripheral blood leukocytes.

Table 11. Distribution of breakages to chromosome groups on rubella patients

chromosome group	long arm	short arm	undifferentiated	total (%)
A 1 2 3	14	1	10 1 4	30 (28.1)
B	17	1		18 (16.8)
C	33	9	2	44 (41.1)
D	10			10 (9.3)
E 16 17 18	1		1	2 (1.9)
F			2	2 (1.9)
G	1			1 (0.9)
total (%)	76 (71.0)	11 (10.3)	20 (18.7)	

Table 12. Distribution of breakages to chromosome groups on control cases

chromosome groups	long arm	short arm	undifferentiated	total (%)
A 1 2 3	8	1	3 4	16 (32.7)
B	7	1		8 (16.3)
C	15	5	1	21 (42.8)
D	4			4 (8.2)
total (%)	34 (69.4)	7 (14.3)	8 (16.3)	

Rubella virus (M-33 strain) was proliferated in a primary culture of GMK cells and 10^{3-4} InD 50/ml. (determined by the titration method in the same GMK cells) was inoculated into a femoral vein in every monkeys. Serum neutralizing titer was examined to confirm the infection. As the controls, chromosome observations were done before the infection.

Method of chromosome preparations in whole blood from African green monkey;

a) Draw 5–10 ml. venous blood from a femoral vein into a sterile syringe with heparin (1 mg./ml.) 1 ml. Pour into a sterile tube and mix.

b) Collect whole blood 3–6 drops and mix with LYG-medium 6 ml. and human AB serum 1.5–2.0 ml. Divide into two TD-15 Earle flasks.

c) Incubate for 60–72 hours at 37° C.

after d), prepared as the same method in human peripheral blood leukocytes.

Results

As seen in Table 13, in all cases from 1 to 5, the incidence of increased

Table 13. Chromosome studies in peripheral blood leukocytes from African green monkeys infected with rubella virus

days after infection	chromosome aberrations (%)				
	case 1	2	3	4	5
before infection	6.6	7.0		0	4.0
1 (days)	14.0			3.7	6.0
2		14.0	27.2	20.0	3.4
3	15.0	22.0			13.3
4		22.0	5.7		12.7
5	12.0	32.0		8.0	12.0
6					
7	18.0			7.6	14.2
8		12.0		6.5	9.5
9		13.3		7.3	7.1
10	0				
11		7.0		11.0	9.0
12					
13					
14	3.3	5.0			
15		10.0		9.0	5.5
total (%)	10.7	15.4	9.5	9.9	8.0

frequency of chromosome breakages over the controls were observed.

Abnormal metaphase plates for 15 days were 10.7, 15.4, 9.5, 9.9 and 8.0 % on an average of each case 1 to 5, being compared with 6.6, 7.0, −, 0 and 4.0 % in controls. Higher chromosome breakages were distributed for the first one week after inoculation, being 18.0 % on the seventh day, 32.0 % on the fifth day, 27.2 % on the second day, 20.0 % on the second day and 14.2 % on the seventh day of the highest for each case. In total cases 11.6 % (204 of 1750 metaphases) of abnormal metaphase plates was shown compared with 5.2 % in controls.

These breakages mostly consisted of chromatid or isochromatid breaks and fragments (96.5 %) with a few interchanges, dicentrics and marker chromosomes (3.5 %) (Table 14). In case 1, 8.6 % of abnormal metaphase plates was noted for two months after the infection. It is supposed from this study that the incidence of chromosome aberrations do not depend on the input multiplicity of the virus.

Table 14. Breakages in peripheral blood leukocytes from African green monkeys (%)

	break or fragment (+) (++) (+++)	interchange	dicentrics	marker chromosome
subjects (%)	83.2 11.8 1.5	1.5	1.5	0.5
controls (%)	100			

3) Chromosome studies in various cultured cell systems infected with rubella virus and adenoviruses.

(1) Chromosome studies on cultured BHK, RE, and GMK cells infected with rubella virus.

Chromosome studies were carried out on cultured BHK, RE and GMK cells infected with rubella virus.

Materials and Methods

Rubella virus had multiplicity of 10^{3-4} InD 50/ml. as the above-mentioned. These tissue cultured cells were grown in MEM (minimal essential-Eeagles) medium supplemented with antibiotics and 2–10 % inactivated calf serum for BHK cell line, in LE (lactalbumin-Earles) medium with antibiotics and 2–5 % calf serum for GMK cell line (for 50 passages) and RE cells (primarily cultured in our laboratory).

In inoculation on each cultured cell systems with comparative growth, usually 12 hours after the infection, virus was permitted to absorb for three hours at 37°C at a concentration of 10^{3-4} InD 50/ml. 0.1–0.2 ml. for 40–50 $\times$ 10^4 cells/ml. 10 ml..

As the controls PBS solution was used instead of rubella virus.

Methods of tissue culture and chromosome preparation;

Tripsinize (10.0 %) the tissue. Float in a 10 ml. culture medium as 40–50 $\times$ 10^4 cells/ml. for primary culture. Incubate at 37° C for adequate times. Strip the cells from culture bottle with 0.25 % trypsin. Drift in a fresh medium. Prepare as the above, for subculture.

Chromosome analyses were prepared according to that of cultured peripheral blood leukocytes, arrested with colchicine for 2 hours, stripped the cells with 0.25% trypsin prior to harvesting. Metaphase plates were counted from 100 to 500 on each sample.

Results

Results were as seen in Table 15 and 16.

a) BHK cells infected with rubella virus.

On samples taken at 12, 24 hours, 2, 7, 10 days after the infection, abnormal metaphase plates were 11.0, 7.0, 17.0, 25.5, 14.0 % respectively in contrast with 6.0, 5.0, 4.0, 8.0, 4.0 % of controls. Abnormal metaphase plates were almost breaks or fragments (82.5 %), and others were diffusely damaged chromosomes, interchanges, rings and marker chromosomes (17.5 %).

b) RE cells infected with rubella virus.

On samples taken at 5, 24 hours, 2, 3, 4, 7 days after the infection, abnormal metaphase plates were 14.1, 10.6, 5.4, 3.8, 0, 1.3 % respectively in contrast

Table 15. Chromosome studies in various cultured cell systems infected with rubella virus

time after virus infection	chromosome aberrations (%)		
	baby hamster kidney cells	rabbit embryonal cells	green monkey kidney cells
3 (hrs.)			12.4 (6.9)
5		14.1 (9.0)	
12	11.0 (6.0)		
24	7.0 (5.0)	10.6 (3.0)	11.4 (8.3)
2 (days)	17.0 (4.0)	5.4 (9.0)	9.2
3		3.8 (2.0)	8.0
4		0	9.5
5			7.0
7	25.5 (8.0)	1.3 (2.0)	
10	14.0 (4.0)		
total (%)	18.3 (6.2)	8.4 (5.0)	9.9 (7.4)

( ); control

Table 16. Breakages in various cultured cell systems infected with rubella virus (%)

	break or fragment (+)	(++)	(+++)	interchange	ring	dicentrics	marker
BHK cells	44.4 (48.9)	30.0 (36.2)	8.1	13.1 (8.5)	3.8 (6.4)		0.6
RE cells	56.3 (100)	26.0		7.7		3.5	6.5
GMK cells	77.7 (78.6)	9.3 (21.4)	0.9	4.6		7.5	

( ); control

with 9.0, 3.0, 9.0, 2.0, –, 2.0 % of controls, being rather higher during 5 to 24 hours. Abnormal metaphase plates were breaks or fragments (82.3 %) and others were diffusely damaged chromosomes, interchanges, dicentrics and marker chromosomes (17.7 %), as compared with 100 % of breaks and fragments in controls.

c) GMK cells infected with rubella virus.

On samples taken at 3, 24 hours, 2, 3, 4, 5 days after the infection, abnormal metaphase plates were 12.4, 11.4, 9.2, 8.0, 9.5, 7.0 % respectively in contrast with 6.9, 8.5, –, –, –, – % of controls, being higher during 3 to 24 hours. Abnormal metaphase plates were breaks or fragments (87.9 %) and others were diffusely damaged chromosomes, interchanges and dicentrics (12.1 %).

(2) Chromosome studies in cultured BHK and HEK cells infected with adenoviruses type 4, 12 and 18.

Chromosome studies were carried out in cultured BHK and HEK cells infected with adenoviruses type 4, 12 and 18. The former cell line is sensitive and the latter is nonsensitive for these viruses. Of these adenoviruses, type 12 and 18 are highly oncogenic and type 7 is weakly oncogenic.

Materials and Methods

Adenoviruses type 4, 12 and 18 were proliferated in primary HEK cells, having a multiplicity of 10^{2-3} InD 50/ml. (determined by titration in the same HEK cells). These two cultured cells were grown in MEM medium supplemented with antibiotics and 2–10 % inactivated calf serum. HEK cells were cultivated primarily in our laboratory from the fetus of induced abortion at the 2nd trimester.

Methods of inoculations, and chromosome studies were carried out in a similar techniques as mentioned before.

Results

a) BHK cells infected with adenoviruses type 4, 12 and 18.

Results were as seen in Table 17 and 18.

On samples taken at 30 minutes to 7 days, abnormal metaphase plates were

Table 17. Chromosome studies in BHK cells infected with Adenovirus type 4, 12 and 18

time after virus infection	chromosome aberrations (%)			
	Type 4	12	18	control
30 min.	10	10	2	2
1 hrs.	8	7	11	6
3	20	10	10	6
6	20	10	14	
18	17	17	37	5
24	16	12	19	3
2 days	13	13	16	0
4	8	10	10	
7	0	10	0	0

Table 18. Breakages in BHK cells infected with Adenoviruses

	break or fragment (+)	(++)	(+++)	interchange	ring chromosome
Type 4	62.8	18.5	6.4	6.8	5.5
12	84.7	10.6	0.9	3.8	
18	91.5	8.0	0.5		
controls	93.2	6.8			

0–10 % for type 4, 7–10 % for type 12 and 0–37 % for type 18 in contrast with 0–6 % of controls, showing moderate difference. Extensive aberrations occured within 24 hours after the infection.

Diffusely impaired cells were noted as 0.5–6.4 % for type 4, 12 and 18.

b) HEK cells infected with adenoviruses type 4, 12 and 18.

Results were as seen in Table 19 and 20.

Table 19. Chromosome studies in HEK cells infected with Adenovirus type 4, 12 and 18

time after virus infection	chromosome aberrations (%)			
	Type 4	12	18	control
3 hrs.	8	0	10	7
18	11	21	14	7
2 days	15	40	14	8
3	8	6	7	3

Table 20. Breakages in HEK cells infected with Adenoviruses

	break or fragment (+)	(++)	(+++)	interchange	ring chromosome
Type 4	85.6	7.8	0.8	5.8	
12	81.4	6.8	1.8	6.5	3.5
18	67.4	19.0	1.8	10.0	1.8
controls	94.7	5.3			

On observations from 3 hours to 3 days, the incidence of abnormal metaphase plates were 8–15 % for type 4, 0–40 % for type 12 and 7–14 % for type 18 in contrast with 3–8 % of controls, indicated increased discrepancy. High aberrations were shown during 18 to 48 hours.

Diffusely impaired cells were noted as 0.8–1.8 % for type 4, 12 and 18.

The results observed here, chromosomal breakages induced with rubella virus and adenoviruses, were similar to those observed in the above-mentioned vivo systems. However diffusely damaged chromosomes such as fragmentations or pulverizations were presented moderately higher in vitro systems.

There were no evident correlations between the types, intensity of damages and oncogenic potentitality of adenovirus type 4, 12 and 18, regardless their oncogenic activity.

DISCUSSION

Since Nichols, et al. (1962) discovered greatly increased incidence of chromosome breakages in cultured peripheral blood leukocytes from measles patients, and Hamper, et al. (1961, 1963) observed increased incidence of chromosome aberrations in Chinese hamster cells infected with herpes simplex virus, one has paid attention to the problems of the virus-chromosome interactions. Past ten years rather many studies of chromosome aberrations in virus-infected cell systems, in vivo and in vitro, have been reported. Now it is apparent that chromosome breakages are induced in virus infected patients such as measles, varicella, rubella, infectious hepatitis, infectious mononucleosis, aseptic meningitis and mumps, in cases of vaccinated patients and also in various cultured cell systems infected with measles virus, rubella virus, herpes virus, SV 40 virus, adenovirus, polio-virus, cytomegalovirus, Sendai virus and mycoplasma, etc. In these studies one attempted to clarify the teratogenic and carcinogenic effects of viruses.

The author observed chromosome aberrations in cultured peripheral blood leukocytes from 197 cases of viral infections of man in cultured peripheral blood leukocytes from African green monkeys infected with rubella virus, and also in various cultured cell systems inoculated with rubella virus and adenoviruses, typically as teratogenic and carcinogenic effects.

As the results, chromosome breakages, increased significantly in frequency, were observed. The most common type of chromosome aberrations observed here consisted of a majority of chromatid or isochromatid breaks and fragments and diffusely impaired chromosomes such as fragmentations or pulverizations, interchanges, dicentrics, rings and marker chromosomes. The frequency of the latter was ranged from a low to a high percentage, in vivo and in vitro systems. The extensive breakages were shown during the fiirst one week at the acute stage in vivo systems, and within 24 to 48 hours relatively at the early stage in vitro systems. The distribution of breakages was proportional to its length of chromosomes such as more than 70–90 % in group A, B, C. D and the long arms of each chromosome and only a few breaks in the smaller groups and short arms of each chromosome in vivo systems.

Some reports showed same results in parts; Nichols, et al. (1962, 1964, 1965) with measles-infected cell systems in vivo and in vitro; Aula (1963, 1964) with varicella; Kuroki, et al. (1966) with rubella; Mella (1968) with infectious hepatitis; Murken, et al. (1968) with measles; Plotkin, et al. (1965) with rubella-infected human tissue culture; Nusbacher, et al. (1967) with congenital rubella syndrome; Stich, et al. (1964, 1968) and Cooper, et al. (1967, 1968) with adenoviruses-infected cell systems, but some parts were different.

Some authors described the divergent results, indicating no increase of chromosome aberrations with viral infections; Tanzer, et al. (1963) with measles; Harnden (1964) and Chun, et al. (1966) with various viral diseases, and Selzer (1963),

Neu, et al. (1964), Valenti (1965), Mellman, et al. (1966), Boué, et al. (1966) and Chang, et al. (1966) with congenital or aborted rubella infections.

Makino, et al. (1966) reported contraversial results in two studies of aseptic meningitis. These may be attributed to the difference of virus strains, individual difference in susceptibility, predisposition, immunity for viruses and to the techniques of chromosome preparations. Kono (1968) pointed out that the difference of frequency of congenital rubella syndrome between U.S.A and Japan might be due to the difference of toxicity of rubella virus in both countries and to the susceptibility of both nations. These questions remain to be solved in the future.

The mechanism of outbreak of chromosome breakages in virus-infected cell systems, has been considered to be impairments of DNA synthesis in direct or indirect virus-cell interactions (Nichols, 1965, 1966, Norrby, et al. 1966). Similar aberrations are induced by ionizing radiations, chemical DNA inhibitors (5-fluolodeoxyuridine, deoxyadenosine, etc.) and antineoplasma agents (actinomycin D, nitrogen mustard, etc.). Some authors have attempted autoradiographic studies (Nichols, et al. 1968, Kato, et al. 1968), enzymological studies (Allison, et al. 1965, Mallucci, et al. 1965), and also the studies using ultraviolet impaired virus (Stich, et al. 1968) to investigate the above-mentioned mechanism.

Clinical connections between chromosome breakages and teratogenesis, carcinogenesis can be found frequently in Bloom's syndrome (Bloom, et al. 1966), Gregg's syndrome (Wiedemann 1944), Fanconi's anemia (Swift, et al. 1966) and congenital telangiectatic erythema (Sawitsky, et al. 1966). The author also experienced 3 cases of aplastic anemia with diffuse chromosome damages in the peripheral blood leukocytes (unpublished data).

Although the mechanism concerning with virus-induced chromosome damages and the clinical appearance due to those chromosome damages has not been become clear, these observations strongly suggest the possible relationships between viral infections and chromosome aberrations, resulting neoplastic transformation and teratogenesis.

With progress in techniques of cytogenetic research, these problems will be solved in detail.

SUMMARY

Chromosome studies were carried out in cultured peripheral blood leukocytes from 197 cases of various viral infections of man in cultured peripheral blood leukocytes from African green monkeys infected with rubella virus (in vivo), and in cultured BHK, RE, GMK cells infected with rubella virus, in cultured BHK, HEK cells infected with adenovirus type 4, 12 and 18 (in vitro).

As the results, 73 of 197 cases of viral infections; rubella, mumps, varicella, measles, viral meningo-encephalitis, viral pneumonia, infectious hepatitis, infectious mononucleosis and herpes zoster patients, showed relatively higher incidence of

chromosome breakages (0–33 %) than the controls (0–7 %).

On 5 cases of African green monkeys infected with rubella virus, abnormal metaphase plates were an average 10.7, 15.4, 9.5, 9.9, 8.0 % as compared with 6.6, 7.0, –, 0, 4.0 % of the controls. The difference was significant.

On BHK, RE and GMK cells infected with rubella virus, abnormal metaphase plates were at 7.0–25.2, 0–14.6 and 7.0–14.6 % as compared with 4.0–8.0, 6.9–8.3 and 2.0–9.0 % of the controls, being slightly or moderately different.

And on BHK cells infected with adenovirus type 4, 12 and 18, abnormal metaphase plates were 0–20, 7–17 and 0–37 % as compared with 0–6 % of the controls, being greatly different. On HEK cells, abnormal metaphase plates were 8–15, 0–40 and 7–14 % as compared with 3–7 % of the controls, being greatly different.

These abnormal chromosomes in present studies were mostly chromatid or isochromatid breaks and fragments with a few to moderate incidence of extensively impaired chromosomes; interchanges, dicentrics, rings and marker chromosomes.

Although the explanation about the relation between cause and effect, viral infection and chromosome aberrations, has not been established, the idea that virus induced chromosome damages will have some connection with neoplastic transformation and teratogenesis is extremly interesting.

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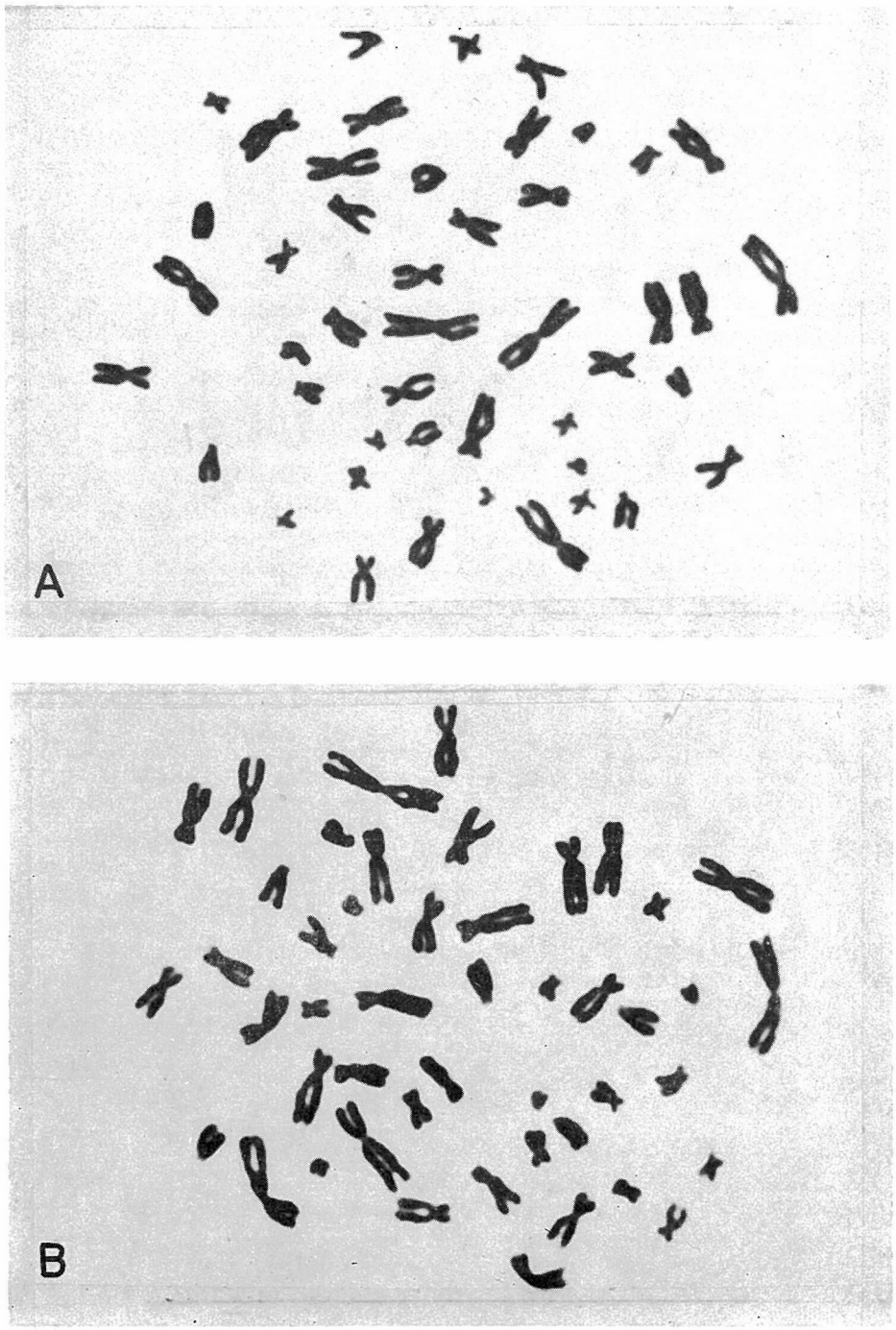

Photo. 1. Normal metaphase plates in male (above) and female (below).

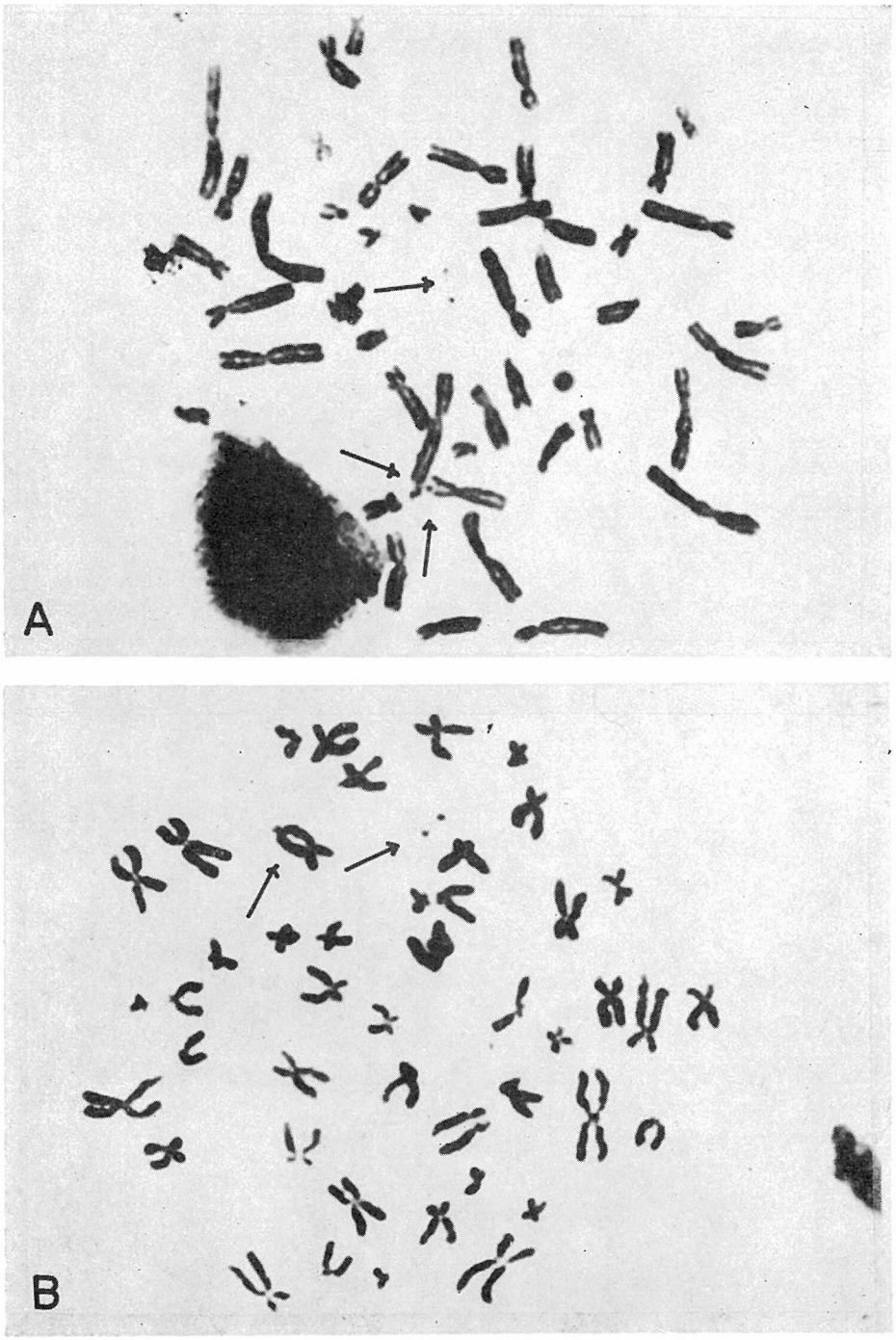

Photo. 2. Abnormal metaphase plates in rubella patients.

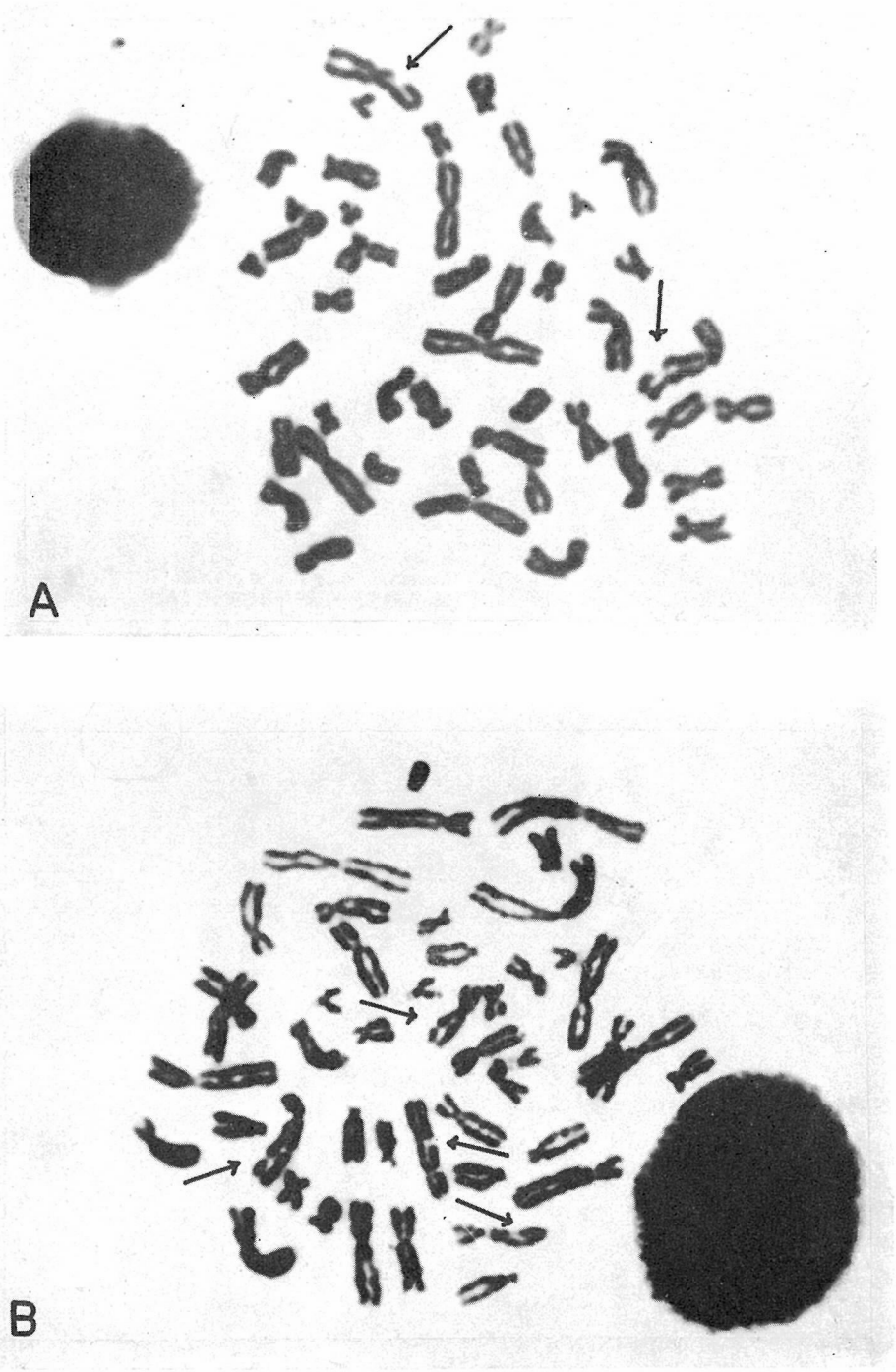

Photo. 3. Abnormal metaphase plates in varicella (above), measles (below) patients.

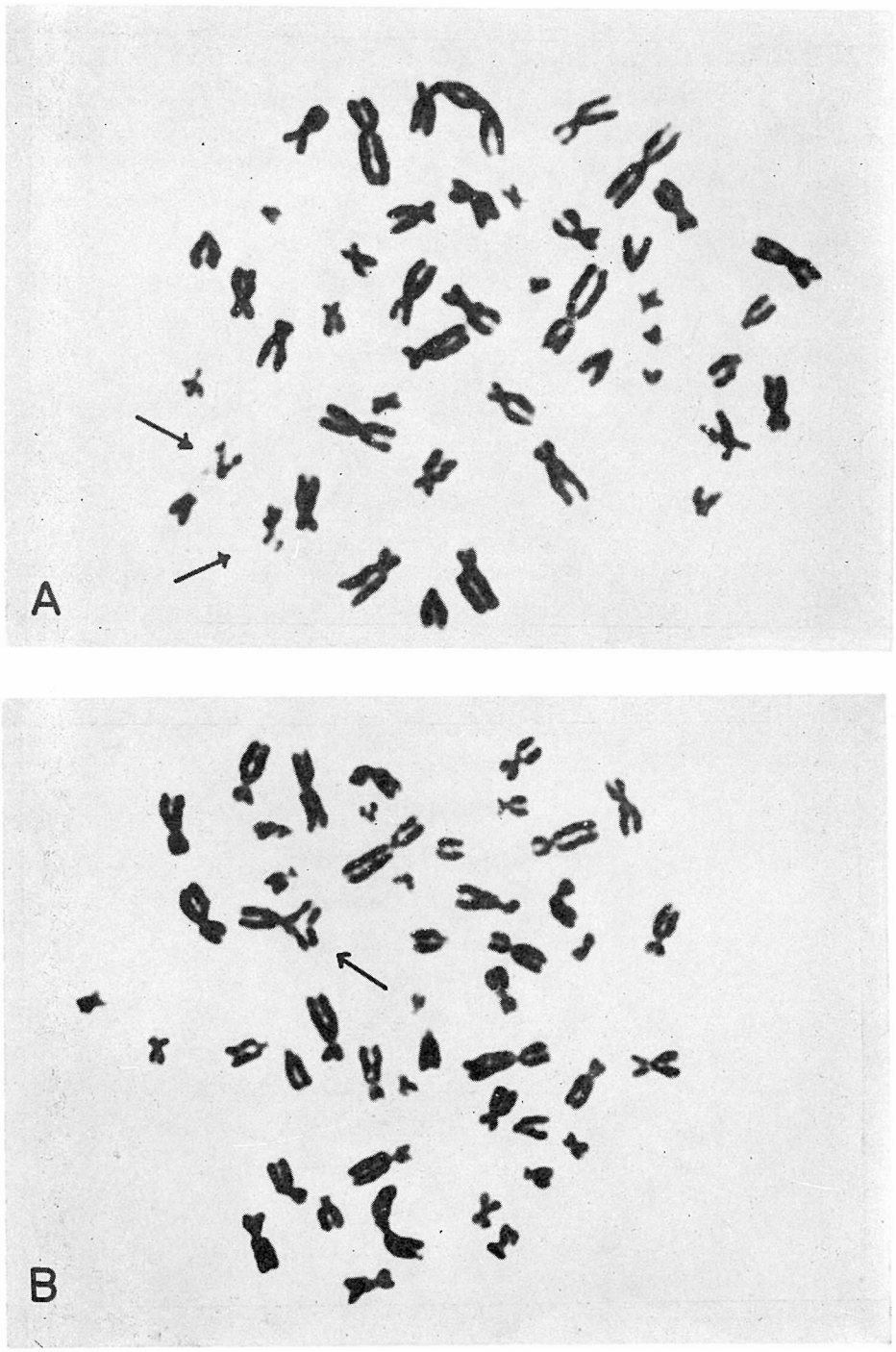

Photo. 4. Abnormal metaphase plates in mumps (above), mumps-meningitis patients.

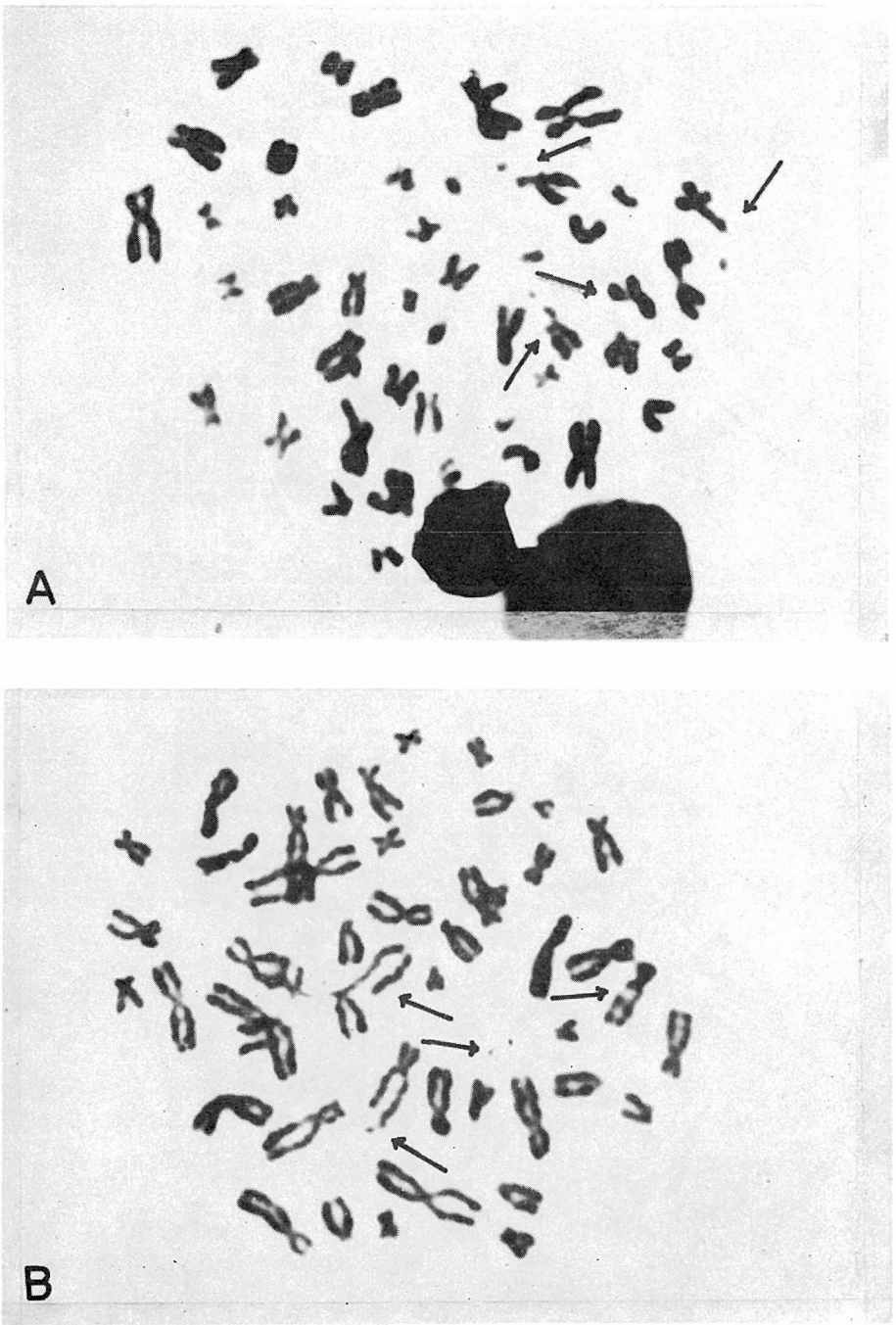

Photo. 5. Abnormal metaphase plates in infectious hepatitis (above), infectious mononucleosis (below) patients.

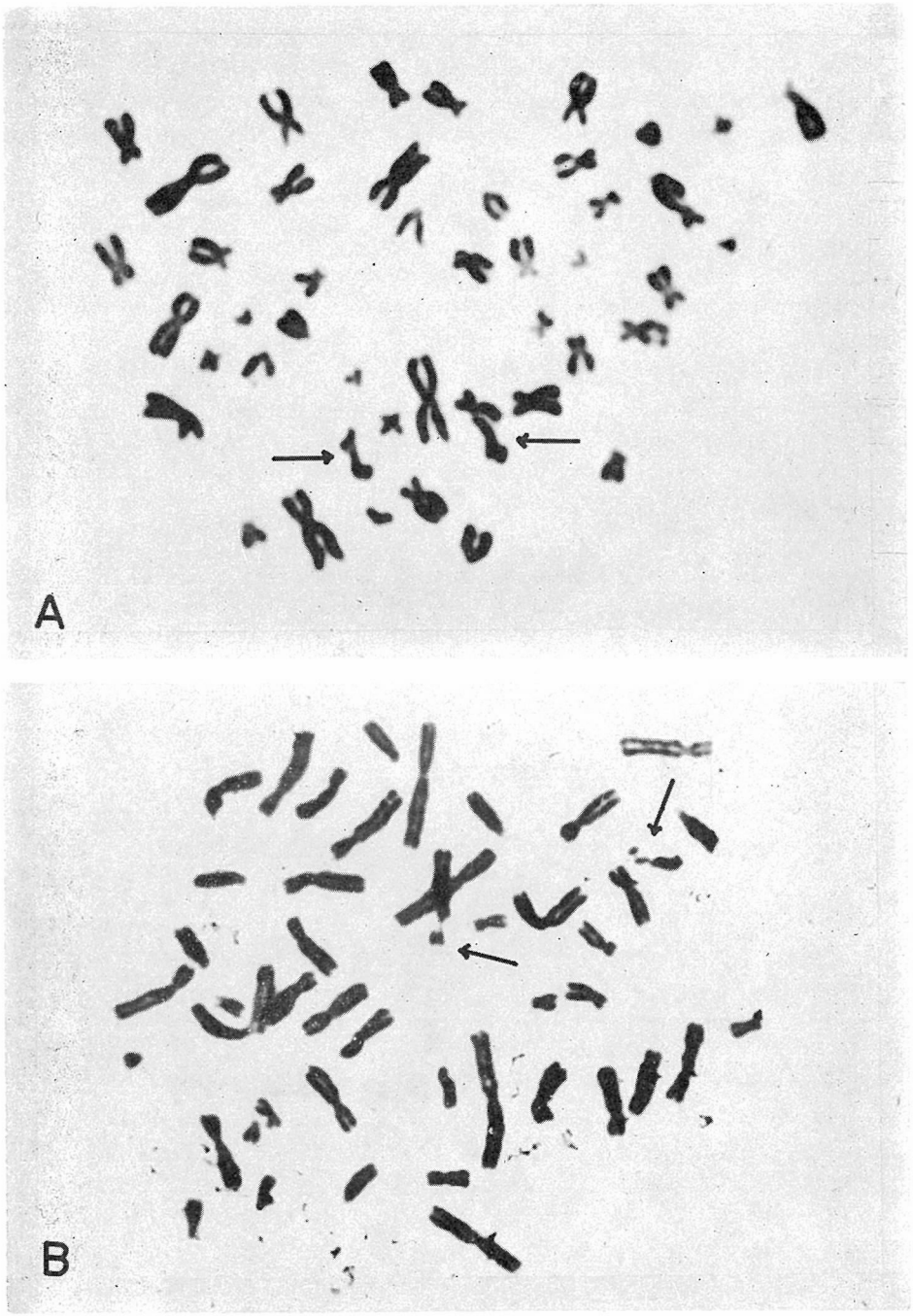

Photo. 6. Abnormal metaphase plates in viral meningitis (above), herpes zoster (below) patients.

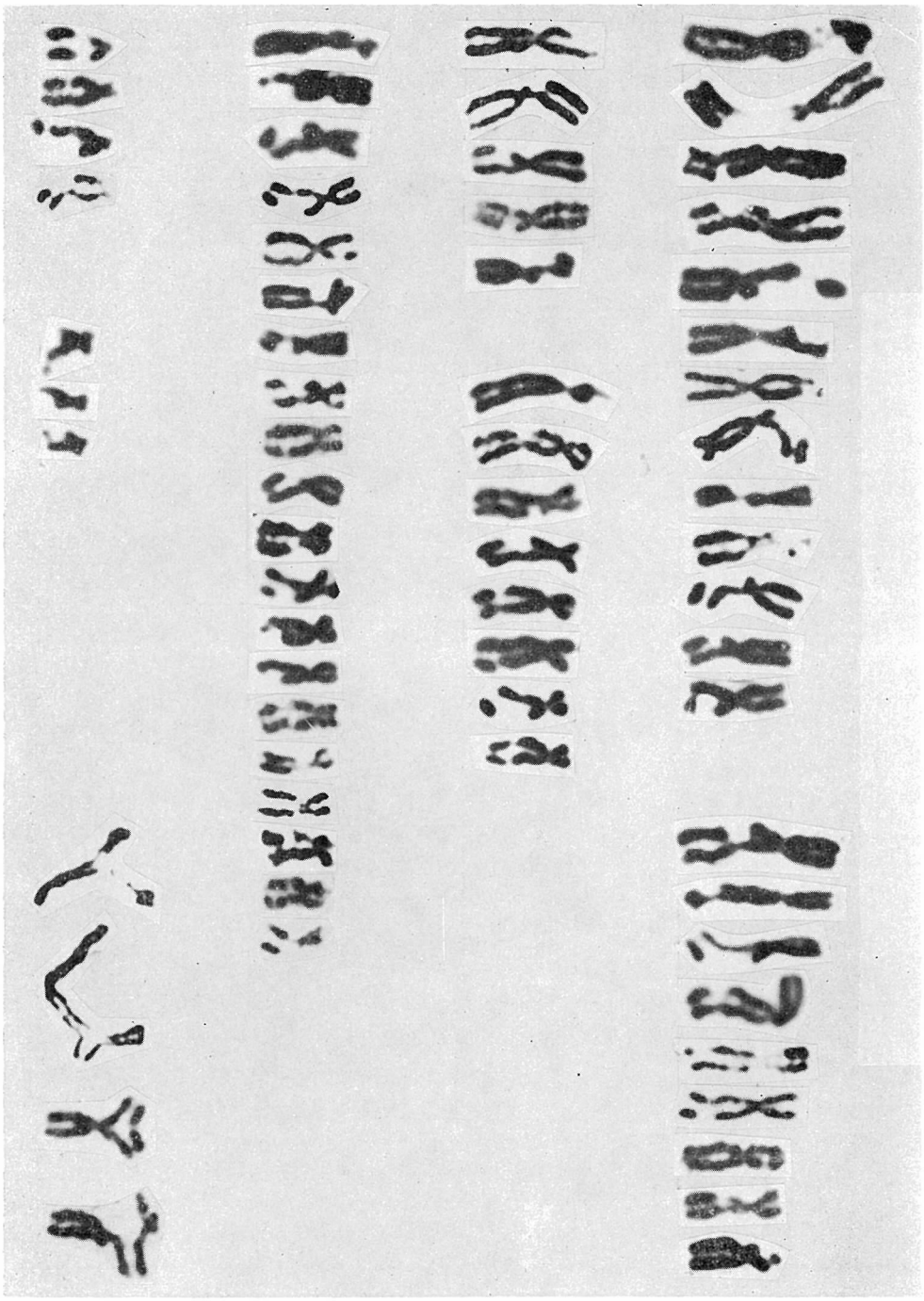

Photo. 7. Various types of breakages in peripheral blood leukocytes from viral patients.

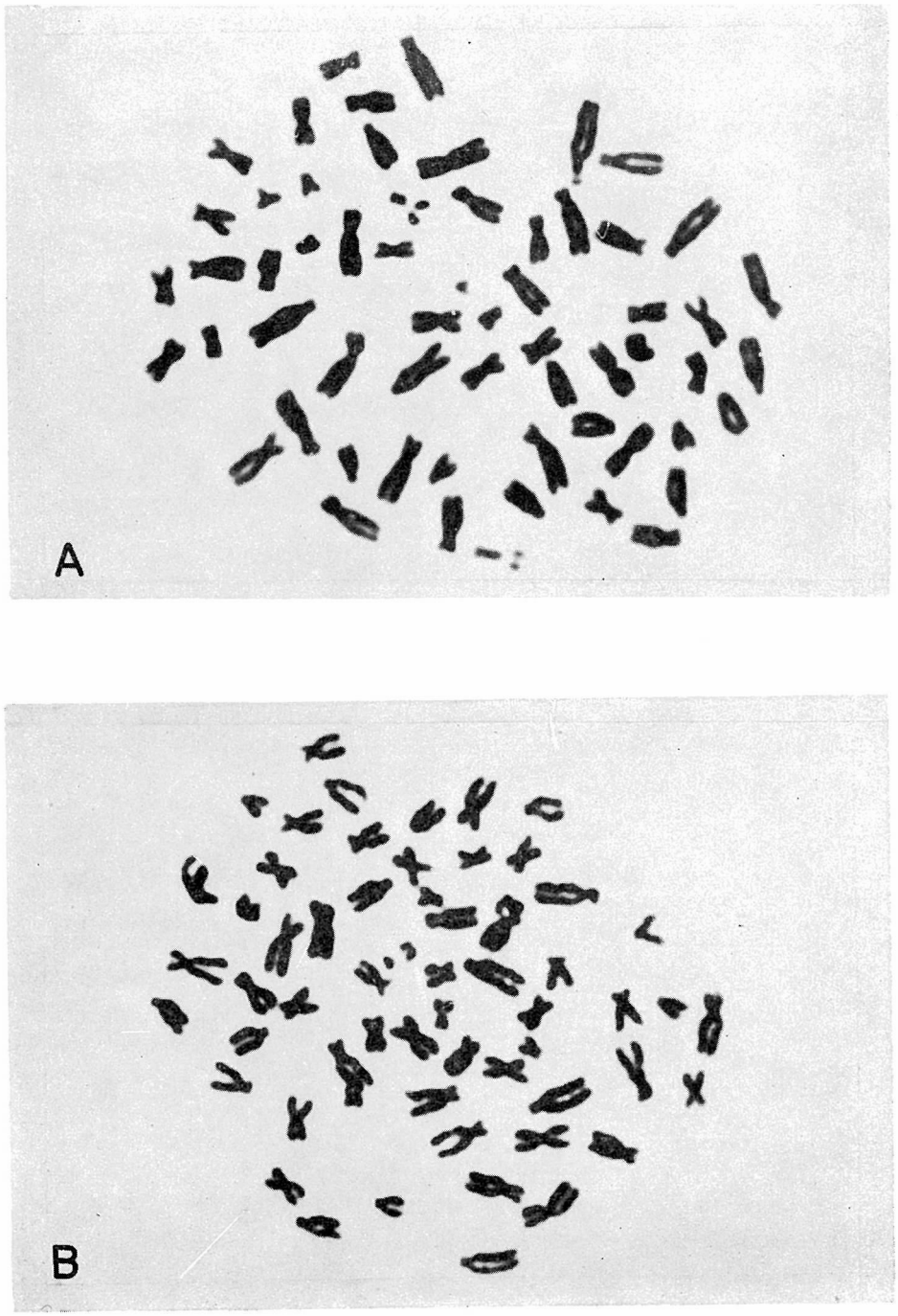

Photo. 8. Normal metaphase plates in peripheral blood leukocytes from African green monkey, male (above) and female (below).

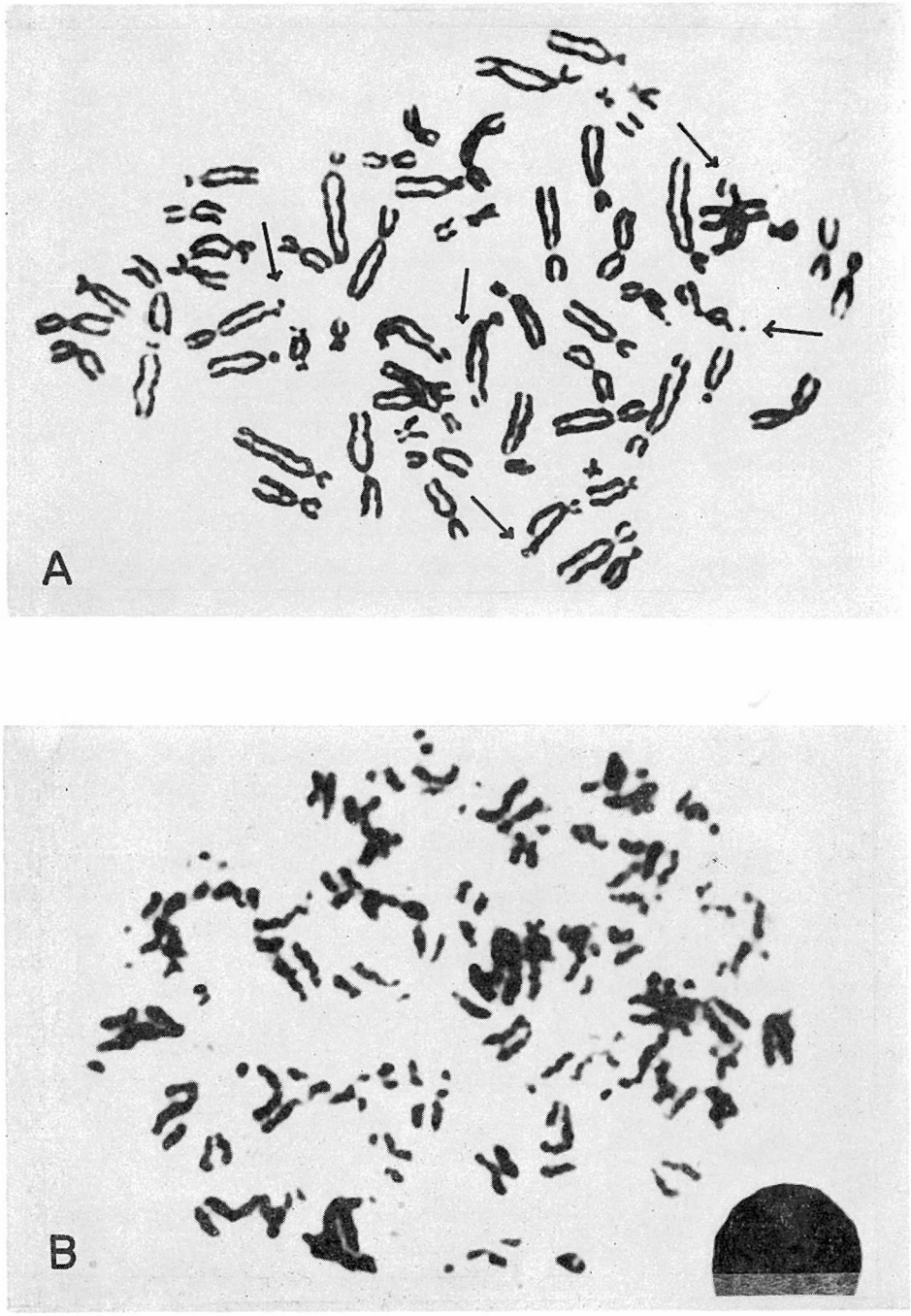

Photo. 9. Abnormal metaphase plates in peripheral blood leukocytes from African green monkey infected with rubella virus.

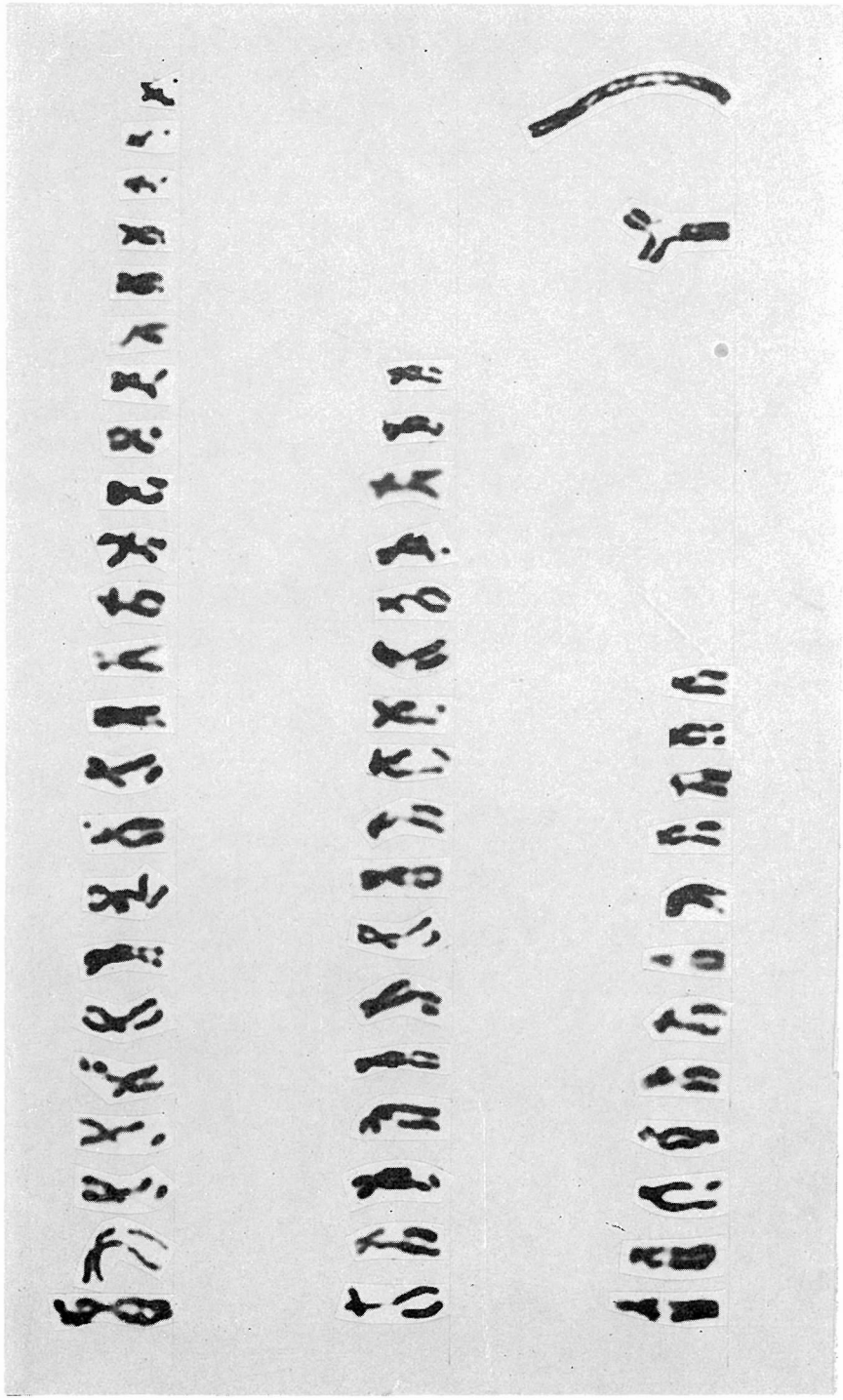

Photo. 10. Various types of breakages in peripheral blood leukocytes from African green monkeys infected with rubella virus.

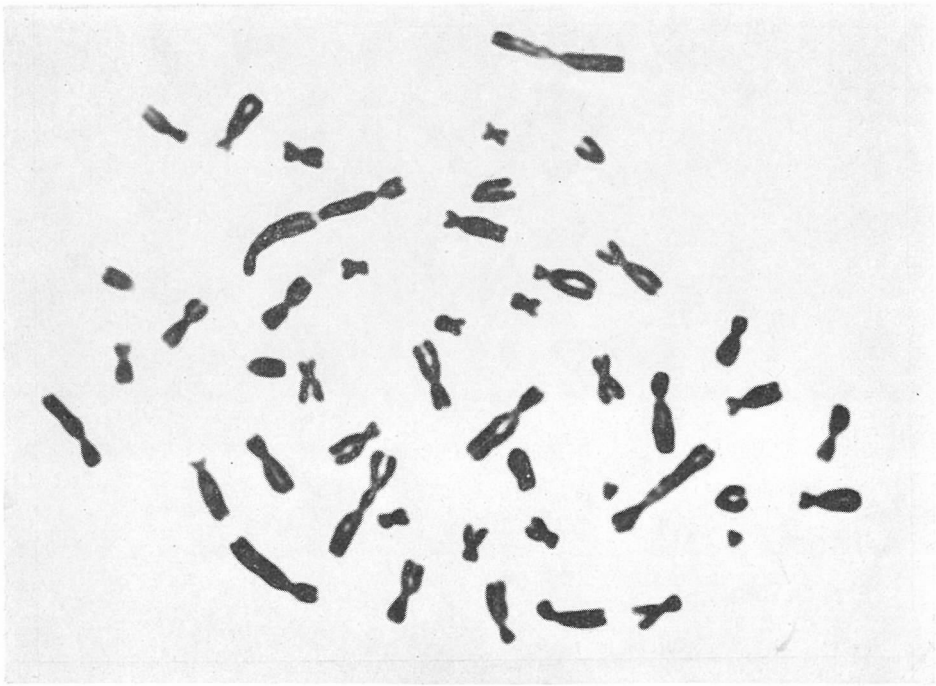

Photo. 11. Normal metaphase plate in cultured BHK cells.

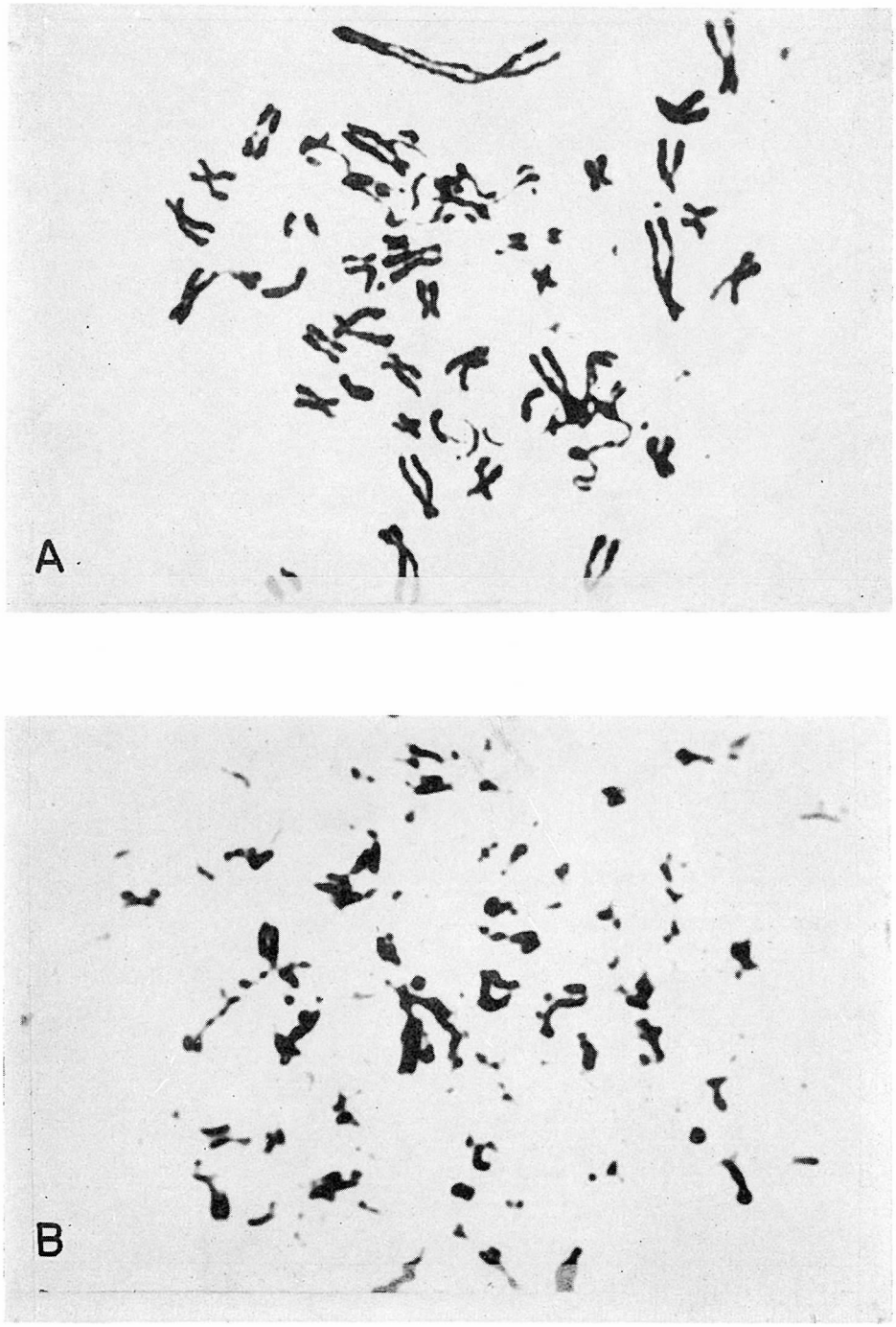

Photo. 12. Abnormal metaphase plates in BHK cells infected with rubella virus.

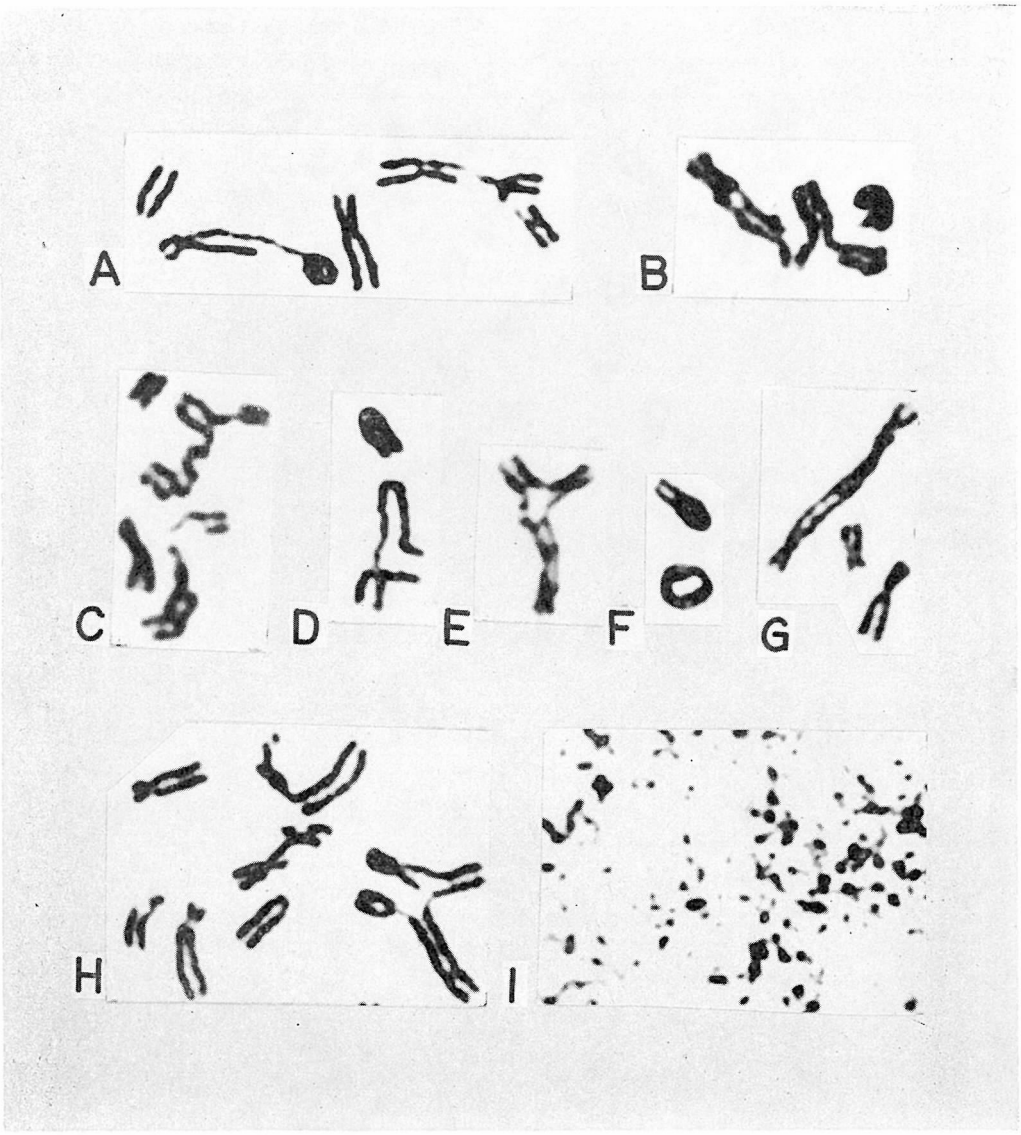

Photo. 13. Various types of breakages induced by rubella virus in BHK cells.

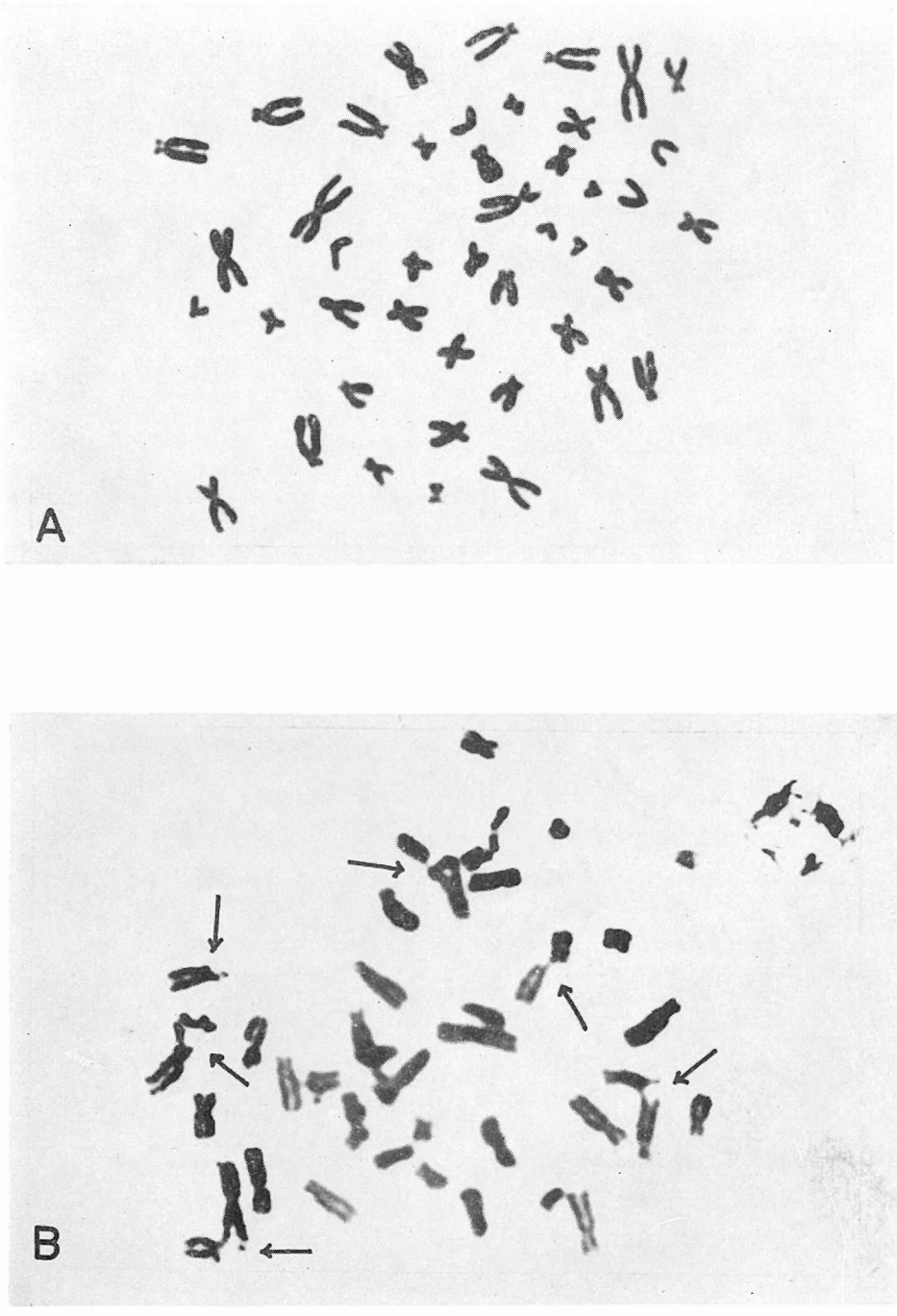

Photo. 14. Metaphase plates in normal non-infected (above) and rubella virus-infected (below) RE cells.

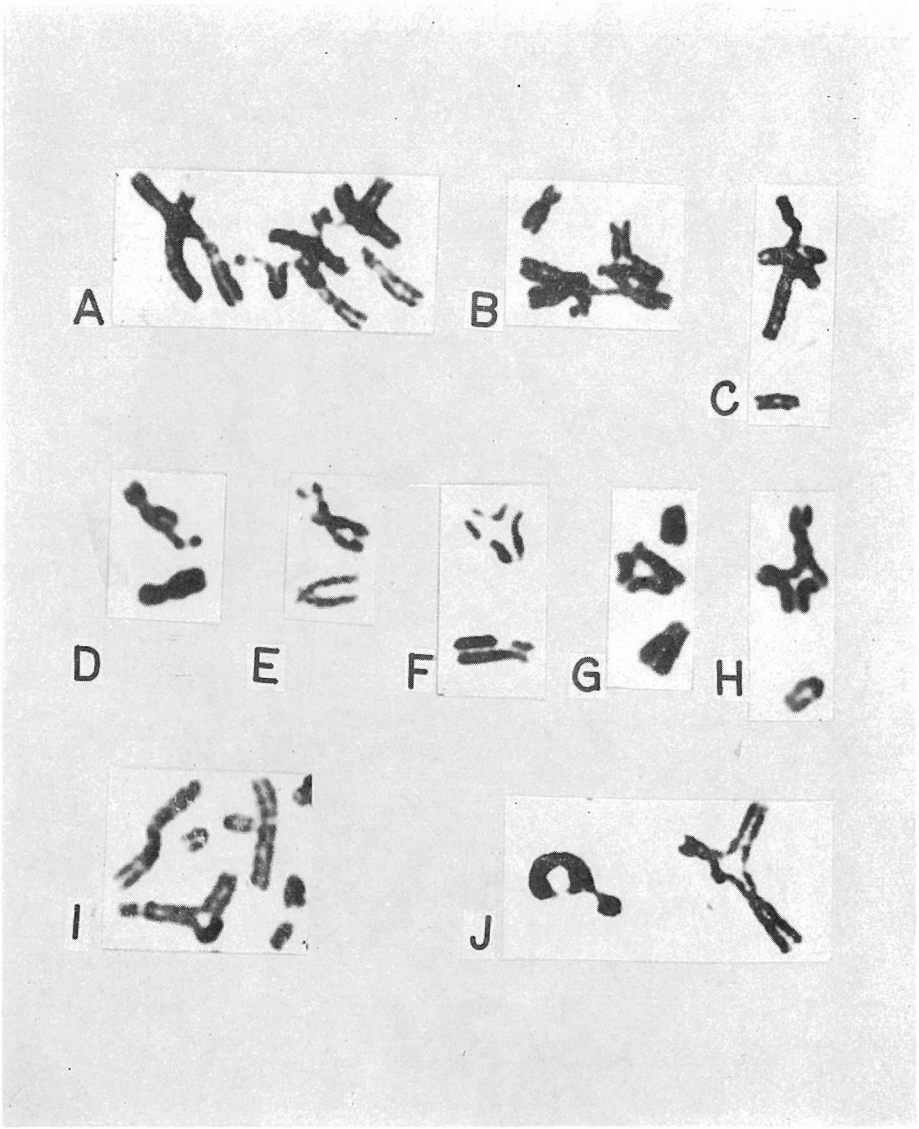

Photo. 15. Various types of breakages induced by rubella virus in RE cells.

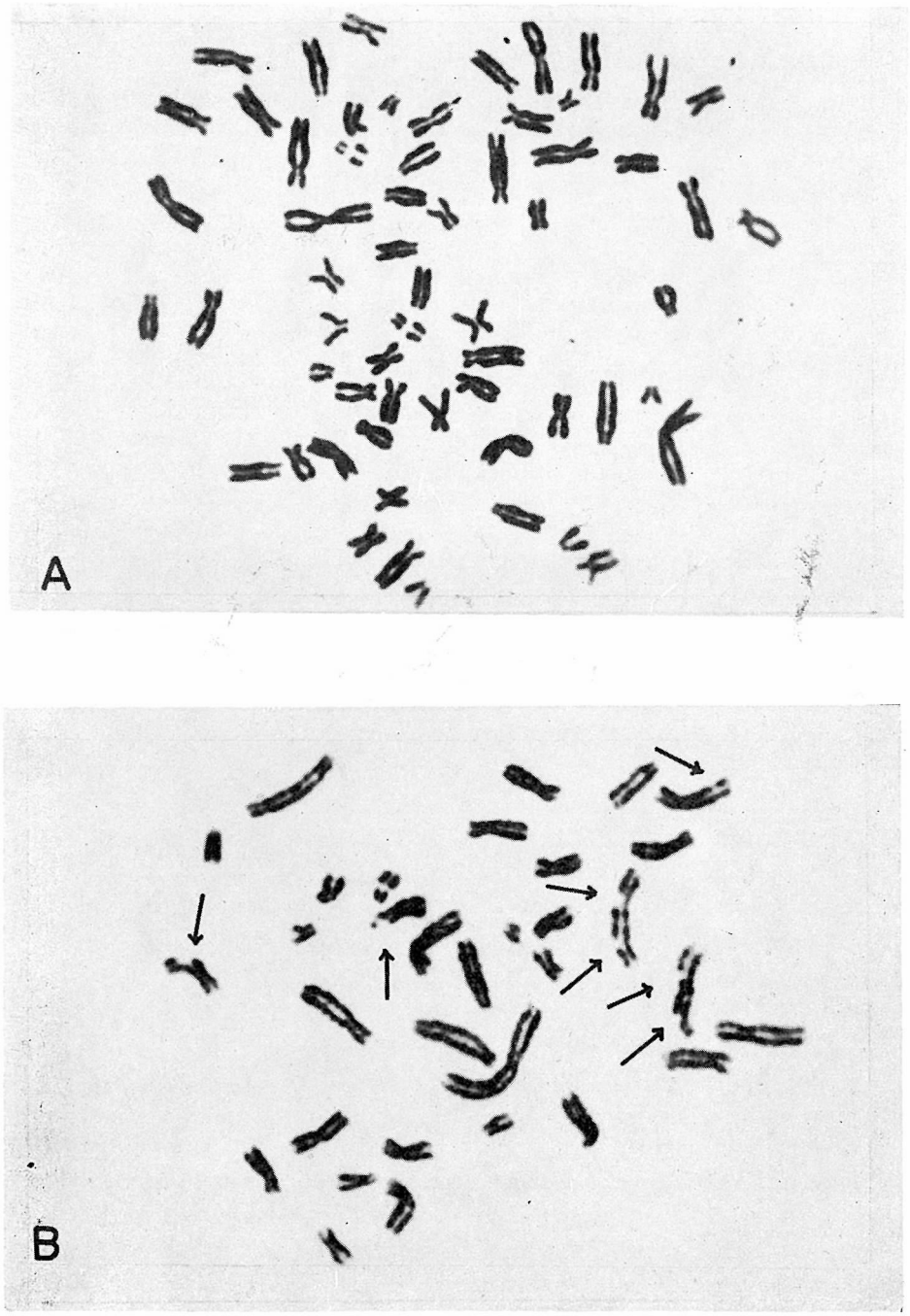

Photo. 16. Metaphase plates in normal non-infected (above) and rubella virus-infected (below) GMK cells.

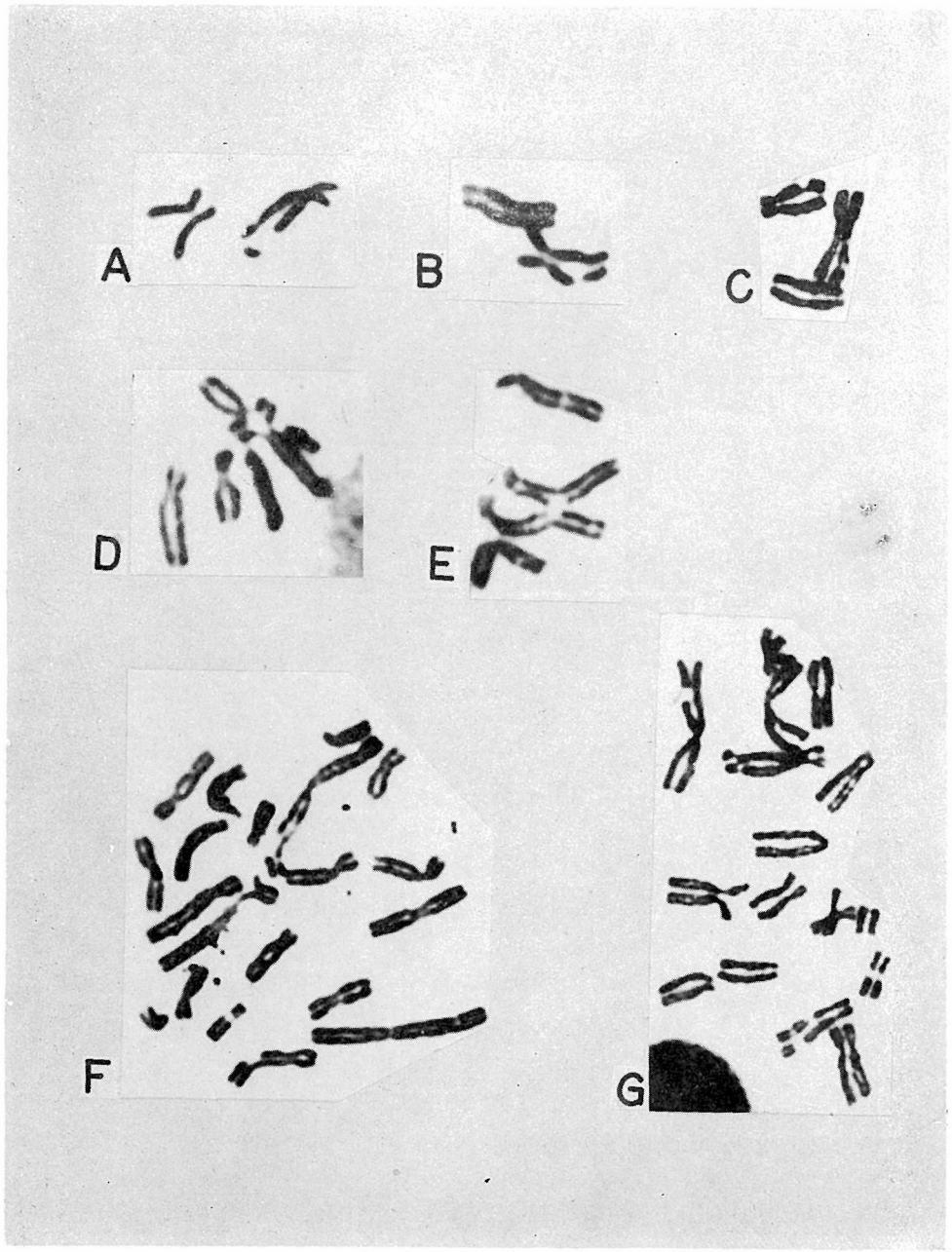

Photo. 17. Various types of breakages induced by rubella virus in GMK cells.

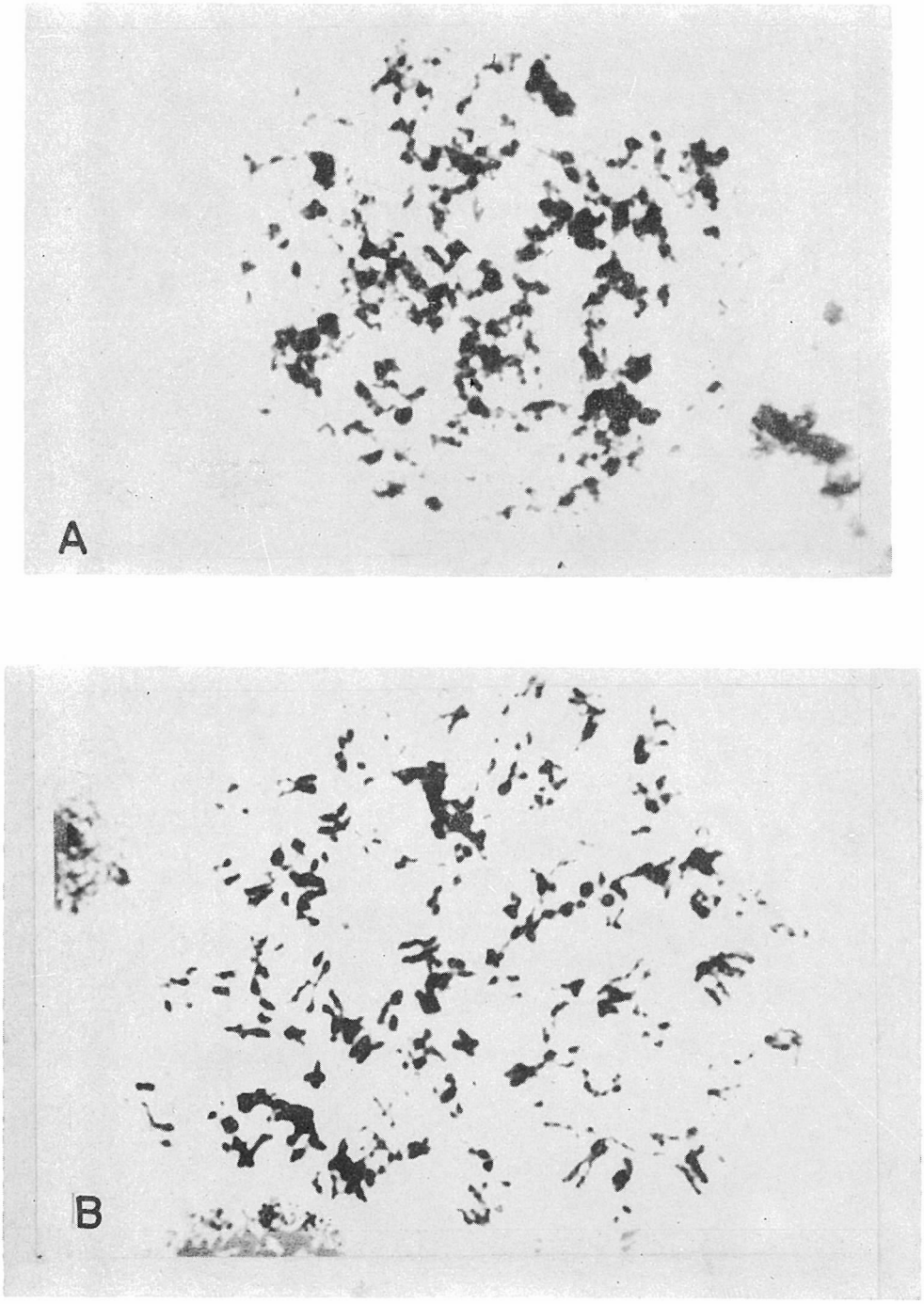

Photo. 18. Abnormal metaphase plates in BHK cells infected with adenoviruses.

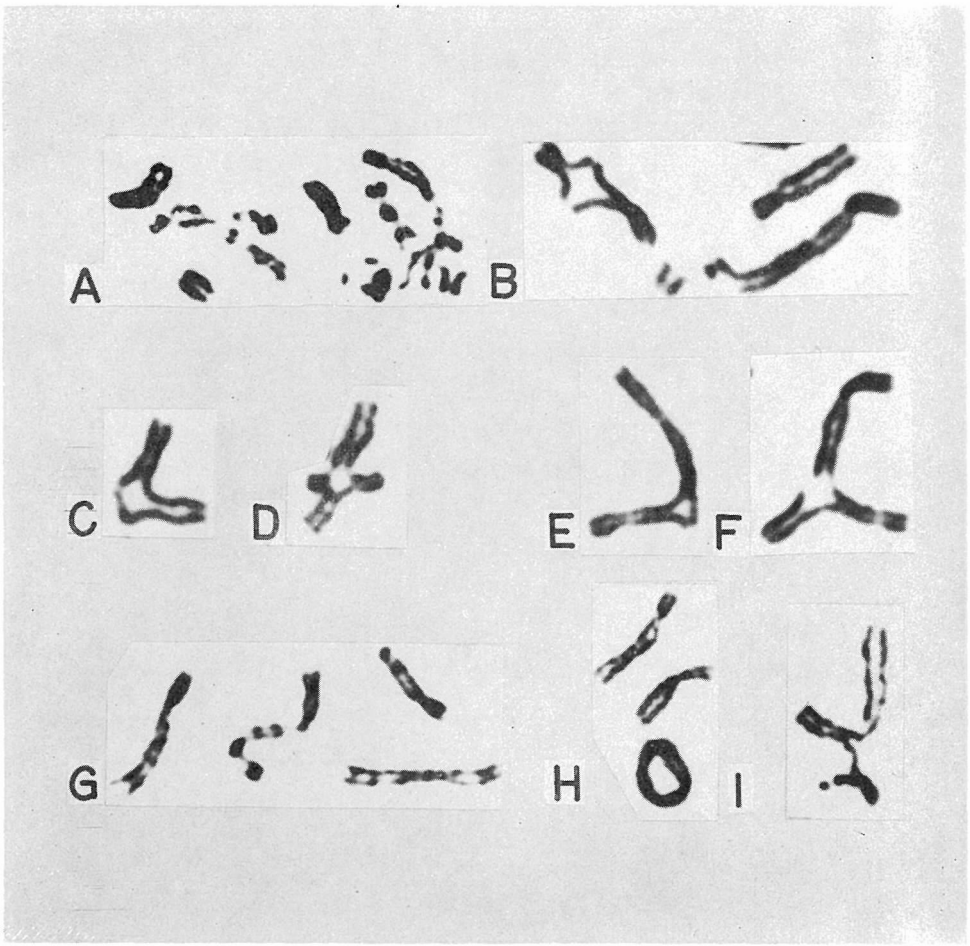

Photo. 19. Various types of breakages induced by adenoviruses in BHK cells.

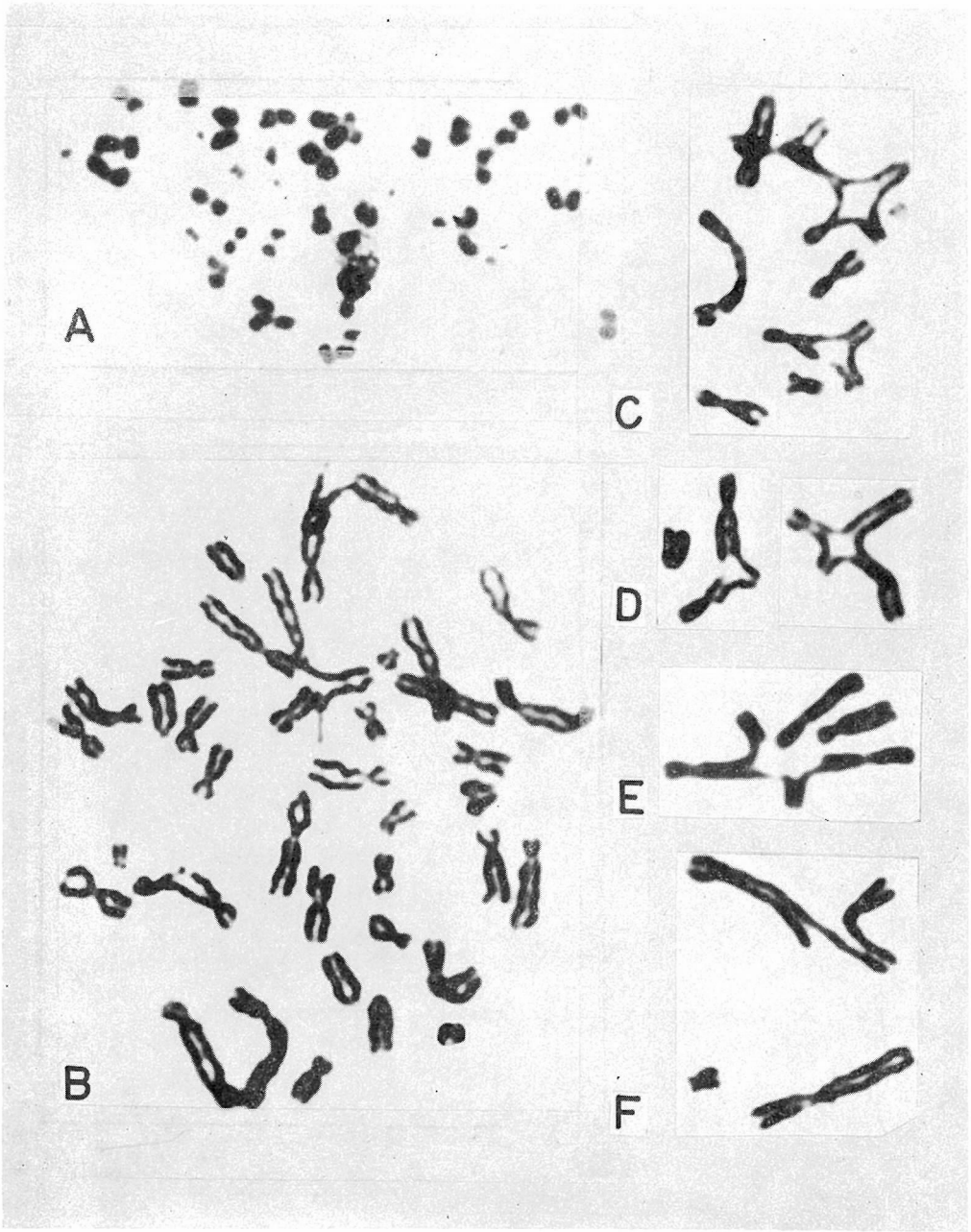

Photo. 20. Various types of breakages induced by adenoviruses in HEk cells.