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STUDIES ON THE CHROMOSOMAL DIFFERENCES AMONG VARIOUS ASCITES HEPATOMAS OF RATS

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APO 343

STUDIES ON THE CHROMOSOMAL DIFFERENCES AMONG VARIOUS ASCITES HEPATOMAS OF RATS

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I. INTRODUCTION

The ascites tumor is an useful tool for the study of chromosomes of cancer. Because, the ascites in this case is actually a suspension of tumor cells and, using a droplet of the ascites, it is very easy to prepare the favorable specimen for the study. However, almost all the ascites tumors available at present are not reproducible and, therefore, they have no analogues enough to be compared with each other concerning their chromosomal pattern.

The ascites hepatoma provides, like other ascites tumors, an useful tool for the investigation of cancer chromosome. This ascites tumor is prepared by means of ascitic conversion of the aminoazodye-induced hepatoma of the rat and as reproducible. The reproducibility is the point which the ascites hepatoma is distinguished from any other ascites tumors. Since 1951 we have already succeeded in establishing 54 different transplant strains of the ascites hepatoma, each strain being derived from different primary hepatoma induced in rats by aminoazodye-feeding, but, naturally all common in the normal ancestral cell, the liver cell of rats.

The comparative studies of different strains of the ascites hepatoma have so far demonstrated that biological characteristics of each tumor strain varied in various aspects such as growth tempo, transplantability (see table 1), ascitic picture and sensitivity to the same chemotherapeutic treatment (1). Among these individual differences of the tumors, most remarkable fact was that each tumor strain differed distinctly each other in the mode of variation of the chromosome number. The range of variation of the chromosome number showed also considerable strain-difference. This was clearly demonstrated by our previous work of this Contract DA-92-557-FEC-31980, as shown in table 2 and chart 1 (2).

The present study aimed at more precise

demonstration of the described chromosomal differences among various ascites hepatomas with regard to the number and the morphological characteristics of chromosomes.

II. MATERIAL & METHOD

1. Ascites hepatomas employed.

Nine different transplant strains of the ascites hepatoma of rats which are maintained by serial animal passages in this laboratory were used. They are strains AH 310, AH 39, AH 423, AH 322, AH 41A, AH 41B, AH 41C, AH 122A and AH 122B. Four strains of them, i.e. AH 310, AH 39, AH 423 and AH 322, derived from different hepatomas induced in separate rats. Two strains, AH 41B and AH 41C, derived from different hepatomas induced in the liver of one rat, while AH 41A came from the spontaneous ascites containing hepatoma cells in the same rat. The remaining 2 strains, AH 122B and AH 122A, derived from the hepatoma and the ascites respectively developed in another rat.

2. Specimen.

The specimen for chromosome examination was prepared as follows; The tumor ascites taken from the ascites hepatoma animal is mixed up in a test tube with a hypotonic NaCl solution (0.03 mol) kept at 37°C. Ratio of the solution to the ascites is 20:1. After giving a good deal of shaking to the test tube, the tube is kept at 37°C. for 10 to 15 minutes. Immediately thereafter, the material is centrifuged in the tube and then stained by addition of large amount of aceto-orcein solution to the pellet. A droplet of this material is placed on a clean glass slide, covered with a cover slip under an adequate pressure, and then sealed with balsam-paraffin. Four-day old tumor ascites exclusively were used for this study, because it was considered that the ascites hepatoma was at the height of its growth in this stage, without being accompanied by significant degenerative processes.

2. Examination of chromosomes.

All metaphase plates of the mitotic ascites

hepatoma cells met with under the microscope were examined, because it was considered necessary not to pick up only preferable nuclear plates but to investigate all metaphase plates in order to study the real entity in the chromosomal features. One hundred in each strain of the tumors, AH 310, AH 39, AH 423 and AH 322, while 50 metaphases were studied in each strain of the remaining tumors, AH 41A, AH 41B, AH 41C, AH 122A and AH 122B. Magnification used for chromosome observation was 100 x 20. All metaphase plates were drawn by the aid of Hamano's camera lucida attached to the one of binocular lens tubes of the microscope used and photographs were taken at the same time in every case by 35mm camera connected to the tube of the same microscope. The mode and the range of variations of chromosome number in each strain were studied and compared with each other. The morphological analysis of chromosomes of each strain was done and chromosomal characteristics were compared with each other.

III. RESULT

1. Chromosome number.

Variations of the chromosome number of nine ascites hepatomas examined in this study were indicated in tables 3-4 and chart 2. There are significant differences in the modal number of chromosomes or the fluctuation range of the 9 tumor strains. The chromosome number of AH 39 fluctuates around 44, the modal number of AH 39, in a relatively narrow range. The remaining 8 strains show respectively different rather wide fluctuation. The modal number of chromosomes is; 71-72 in AH 310, 66-67 in AH 423, 68 in AH 322, 54 in AH 41A and AH 41B, 52-53 in AH 41C, 76 in AH 122A and 71 in AH 122B. Between AH 41A and AH 41B there seems no distinct difference in the modal number of chromosomes, however, the range of variations of the chromosome number differs slightly. The modal chromosome number of all the strains examined is different from the accepted regular number of chromosomes in the rat, 42(=2n).

It may be noteworthy that every strain presents only one mode, without suggesting any possiblity of polymodal variations of the chromosome number.

2. Chromosome morphology.

The ascites hepatoma cells contain various sized chromosomes which differ in shape. The main types of them which are most frequently met with are telocentric, subtelocentric and metacentric ones. But, in some strains of the ascites hepatoma most tumor cells have prominent chromosomes of characteristic features which are not found in normal cells of the rat. Ideograms of chromosomes of 15 ascites hepatomas including the present 9 strains were shown in figures 1 and 2. Predominantly large metacentric chromosomes appeared in only 4 tumors among the 15 ascites hepatomas and the number of chromosome of this type varied in each strain of the 4 tumors. The number is; 1 in AH 423, 2 in AH 66F

and 3 in AH 13 and AH 99. One, two or three large chromosomes with second constrictions were found in AH 423, AH 62, AH 310, AH 62F, AH 99 and AH 414. One minute chromosome was found in AH 310, AH 322 and AH 41B. One satellite chromosome was detected in AH 310. The minute or satellite chromosomes have never been described in ascites tumor cells of the rat. The nuclear plates including these chromosomes were illustrated in figures 3 and 4.

These observations may support the concept of individuality of tumors that each tumor has its own chromosomal patterns.

IV. SUMMARY & CONCLUSION

The ascites hepatoma, prepared by ascitic conversion of the aminoazodye-induced hepatoma of the rat, can provide a series of ascitic tumors for comparative studies on a variety of tumors of a common normal ancestral cell, the liver cell.

In 9 different transplant strains of the ascites hepatoma, the chromosome was studied with special respects to the mode of chromosome number and chromosome morphology and compared with each other. Significant differences were noted in the modal chromosome number and fluctuation range of chromosome number as well as chromosome features.

This may mean that each tumor, originating from a common normal ancestral cell, has its own chromosomal pattern.

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Gann 51:88, 1960

Table 1

Transplantation Rate and Survival Days

33 Strains

Ascites Hepatoma

Strains	Established in	Transplantation		Survival Days
		Generation	Rate(%)	
AH 130	1951	440	96.7	12
7974	1952	420	96.5	12
601	-	310	87.0	17
602	-	330	93.6	18
66	1954	260	91.6	12
63	1955	220	81.5	14
149	-	230	93.0	12
39	-	220	87.7	16
49	-	250	85.7	13
99	1956	230	91.8	11
66F	-	230	87.6	10
322	1957	200	83.9	11
414	-	170	78.5	16
21	-	210	91.3	8
318	-	200	93.0	8
423	-	180	68.2	16
13	-	230	95.5	8
62	-	170	94.0	12
173	1958	160	85.8	11
408	-	140	85.0	12
310	-	130	84.8	13
311	-	140	79.0	12
272	-	180	97.8	7
286	-	130	84.2	12
127	-	90	93.8	17
62F	-	50	78.8	18
44	1959	60	82.2	10
34	1960	60	94.2	16
41A	-	43	94.0	12
41B	-	44	89.0	12
41C	-	41	93.0	10
122A	1961	32	97.0	13
122B	-	32	95.0	17

Table 2

Chromosome Number of Ascites Hepatomas
AH 130, 7974, 601, 602, 66, 13,
99, 414, 62F, 62 and AH 66F
(Examined on 4-day-old Tumors)

Strain	No. of nuclei examined	Mode	Range of variations
AH 130	100	43	38-47
AH 7974	100	49	44-54
AH 601	100	67-68	63-75
AH 602	100	67-68	60-76
AH 66	100	70	62-78
AH 13	100	38	36-39
AH 99	100	64	61-67
AH 414	100	47-48	44-51
AH 62F	100	48	45-51
AH 62	100	67	64-70
AH 66F	100	38	35-41

Table 3

Distribution of Chromosome Number of the Ascites Hepatoma AH 310, AH 39, AH 423 and AH 322 (Examined on 4-day-old Tumors)

No. of Chromosome	Frequency				No. of Chromosome	Frequency			
	AH 310	AH 39	AH 423	AH 322		AH 310	AH 39	AH 423	AH 322
	* 117th	* 212th	* 175th	* 194th		* 117th	* 212th	* 175th	* 194th
35		1			69	7		2	9
38		1			70	13		6	8
41		2			71	15		2	5
42		9			72	12		1	4
43		11			73	13		1	
44		39			74	7		1	5
45		21			75	7			1
46		8			76	4			
48		1			77	1			1
50	1				78	1			2
51		1			79	1			
53		3			80	1			
56	1			1	88		3		
57				1	92				2
59	1				101	1			
60			1	1	104				1
62	1		1	1	122			1	
63	1		5	2	132				1
64	2		14	1	136	1			
65			13	10	137				1
66	2		18	12	146	1			
67	2		18	13	Total	100	100	100	100
68	5		16	18					

* Generation of serial transfers of tumors at the time of chromosome examination.

Table 4

Distribution of Chromosome Number of the Ascites Hepatoma AH 41A, AH 41B, AH 41C, AH 122A and AH 122B (Examined on 4-day-old Tumors)

No. of Chromosome	Frequency				
	AH 41A	AH 41B	AH 41C	AH 122A	AH 122B
	20th*	15th*	15th*	12th*	16th*
42	1				
46	1		1		
47	1		1		
48	1		3		
49	1		1		
50	1	4	7		
51	6	5	8		
52	6	7	7		
53	7	4	9		
54	8	10	7		
55	5	3	2		
56	4	5	1		
57	1	6			
58	3	2			
59		1			
61	2	1		1	2
63		1			
65				1	1
66	1			2	1
67					1
69				1	3
70					3
71				1	11
72				3	6
73				1	6
74				5	1
75		1	1	8	4
76				11	5
77				3	1
78				3	
79				5	
80				3	1
81				1	1
83					1
84	1			1	
87					1
90				1	
97			1		
110					1
111			1		
Total	50	50	50	50	50

* Generation of serial transfers of tumors at the time of chromosome examination.

Chart 1
The Ascites Hepatoma
Distribution of the Chromosome Number

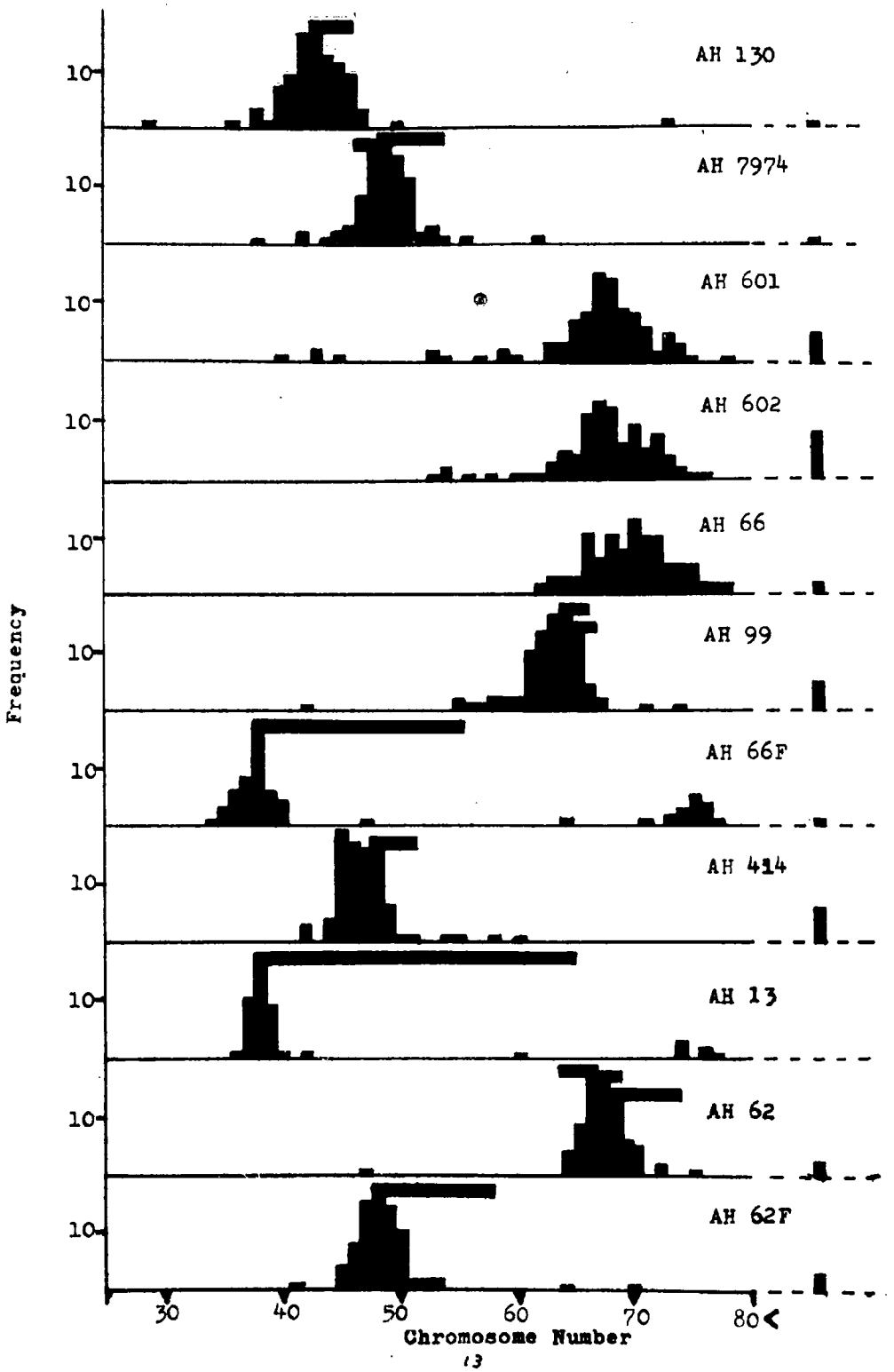
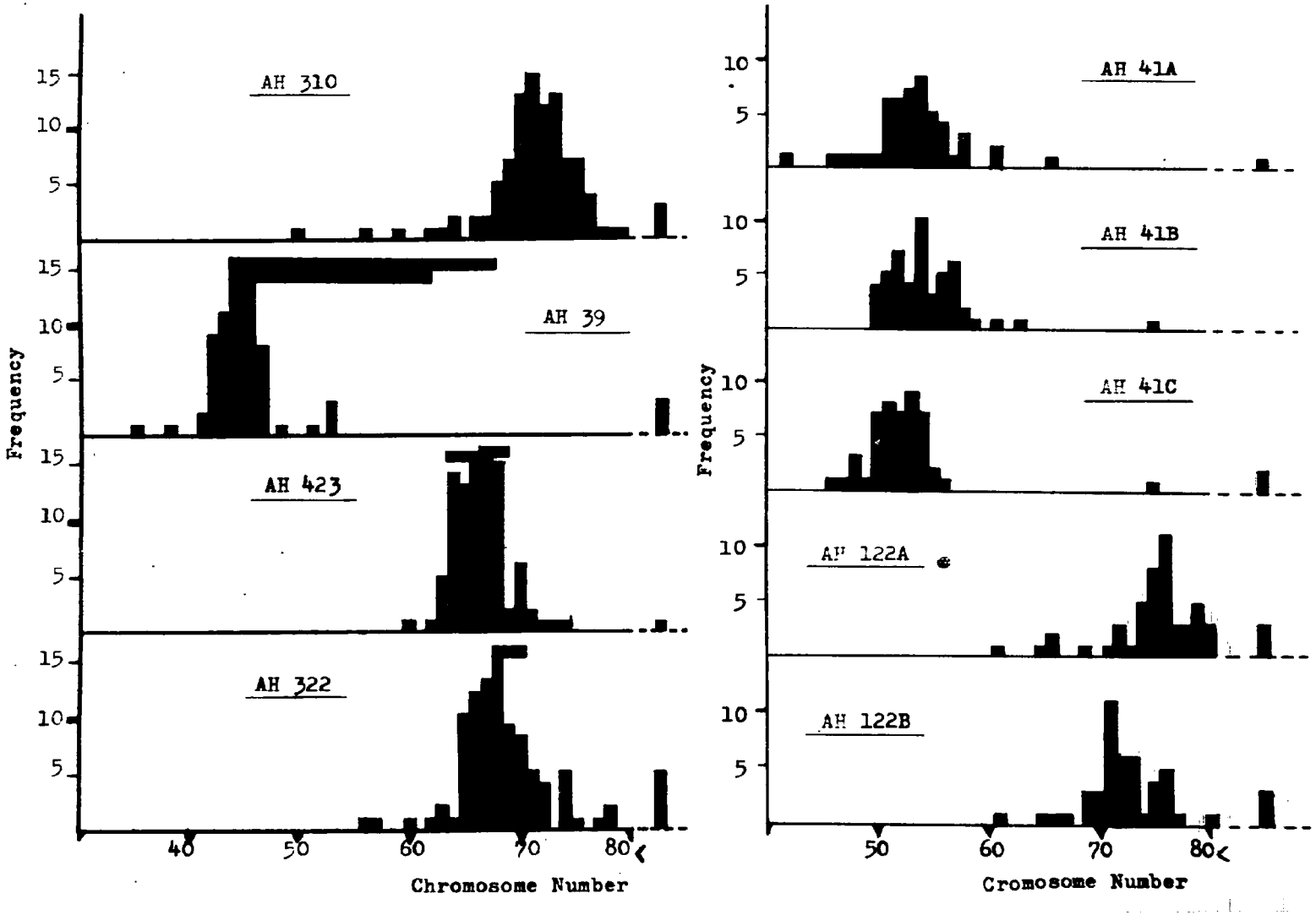


Chart 2

The Ascites Hepatoma

Distribution of the Chromosome Number



EXPLANATION OF FIGURES

Fig. 1. Chromosomes of 10 different ascites hepatoma cells of the rat. AH 423, 66 chromosomes. AH 13, 38 chromosomes. AH 62, 67 chromosomes. AH 310, 72 chromosomes. AH 62F, 47 chromosomes. AH 39, 44 chromosomes. AH 99, 64 chromosomes. AH 66F, 38 chromosomes. AH 322, 68 chromosomes. AH 414, 47 chromosomes.

Fig. 2. Chromosomes of 5 different ascites hepatoma cells of the rat. AH 41A, 54 chromosomes. AH 41B, 54 chromosomes. AH 41C, 52 chromosomes. AH 122A, 74 chromosomes. AH 122B, 71 chromosomes.

M, large metacentric chromosome. m, minute chromosome. S, satellite chromosome. C, chromosome with a second constriction.

Figs. 3 and 4. Examples of photomicrographs of metaphase plates of ascites hepatoma cells. **Fig. 3;** AH 310, chromosome number 72. Note a minute chromosome (m), a satellite chromosome (S) and chromosomes with second constrictions (C). **Fig. 4;** AH 423, chromosome number 66. Note a large metacentric chromosome (M) and a chromosome with a second constriction (C).

Figure 1

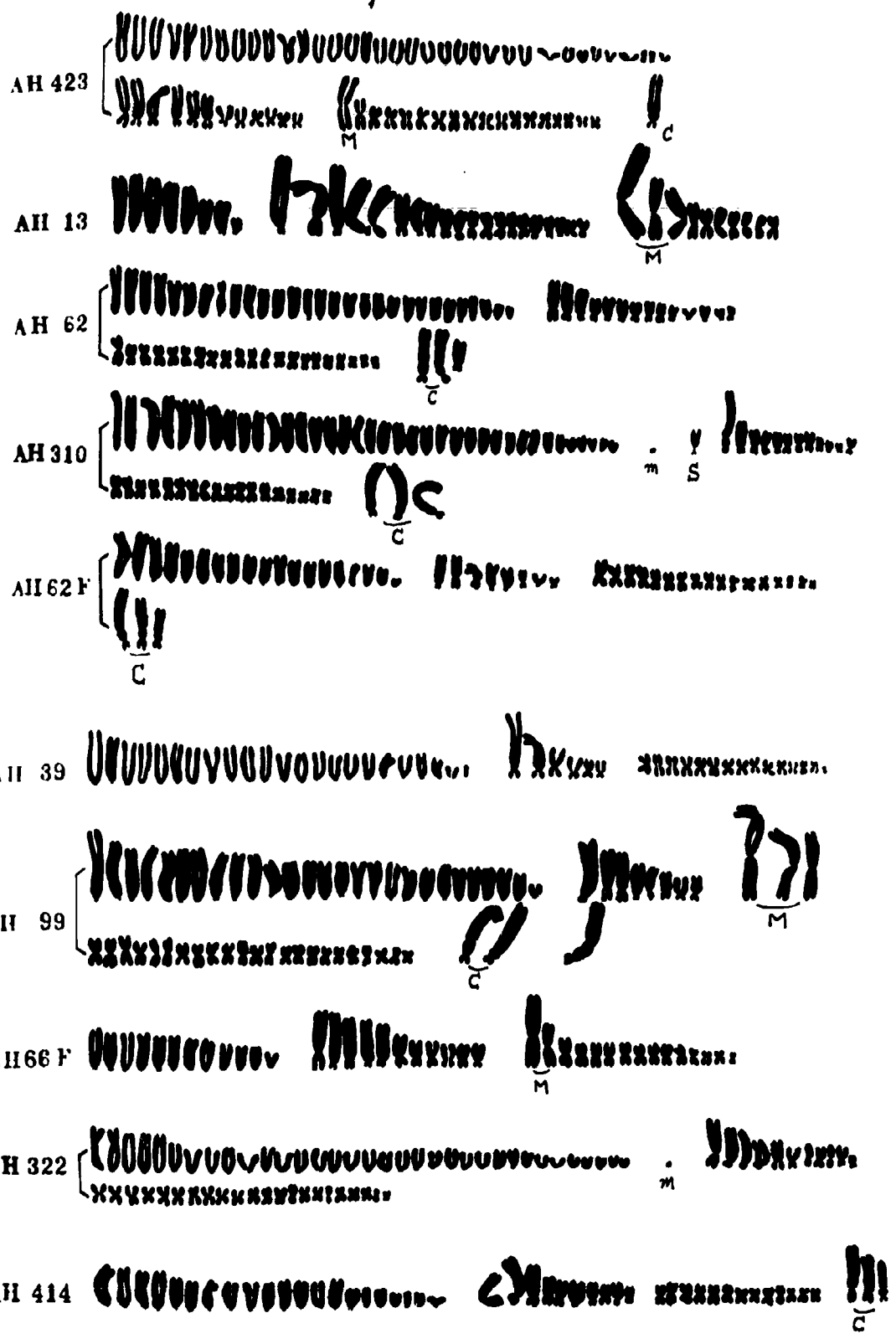
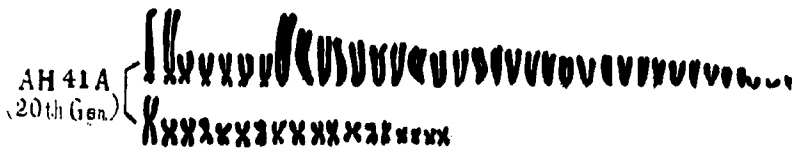

AH 423
M
C
AH 13
M
AH 62
C
AH 310
m
S
C
AH 62 F
C
AH 39
AH 99
M
C
AH 66 F
M
AH 322
m
AH 414
C

Figure 2

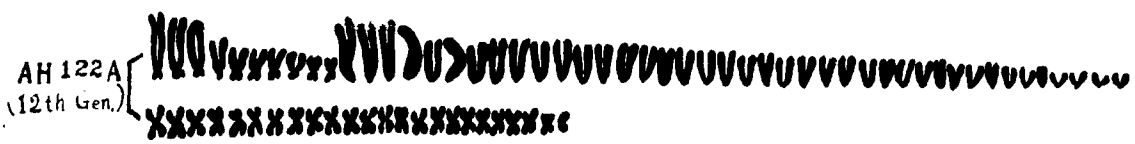
AH 41A (20th Gen.)



AH 41B (15th Gen.)

AH 41C (15th Gen.)

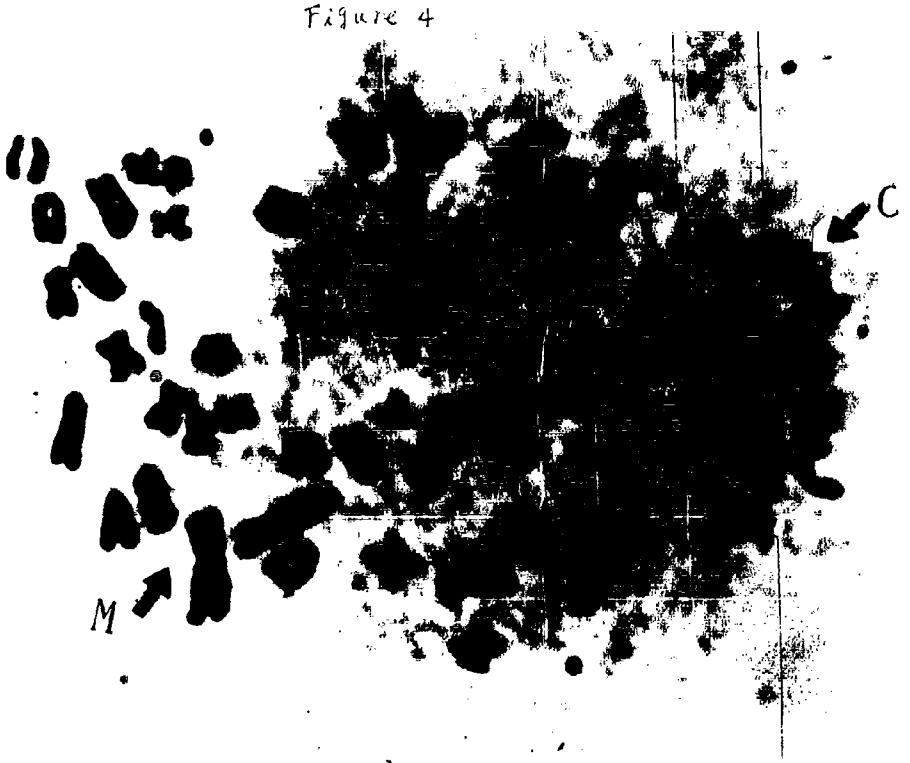
AH 122A (12th Gen.)



AH 122B (16th Gen.)

Figure 3

Figure 4



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