Chromosomal Alteration and Development of Tumors. XIX. Chromosome Constitution of Tumor Cells in 16 Plasma Cell Neoplasms of BALB/c Mice[1,2]

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SUMMARY—Karyotypes of 16 plasma cell neoplasms induced by mineral oil or mineral oil adjuvants in BALB/c mice were analyzed. Of these neoplasms, 13 produced specific immunoglobulins, 13 had stemlines characterized by hypotetraploid chromosome numbers, 1 (MOPC-31C) had a hyperdiploid stemline, 1 (MOPC-70E), a hypotriploid stemline, and 1 (Adj PC-6C), a hypertetraploid stemline. The stemline cells of 13 neoplasms had 1–4 marker chromosomes, whereas those of 3 neoplasms (MOPC-31B, MOPC-70A, and MOPC-70E) had no detectable marker chromosomes. The wide range of chromosome numbers usually found in the early transplant generations narrowed in the later transplant generations. Three neoplasms not producing specific γ-immunoglobulins evolved from 3 neoplasms characterized by the production of γA serum, lambda chain and γF serum, and excess kappa, respectively. The karyotypes of these negative neoplasms differed significantly from those of the parental positive neoplasms.—J Nat Cancer Inst 41: 1083–1097, 1968.

PLASMA CELL TUMORS induced by intraperitoneal injections of mineral oil (*1*) in the highly inbred BALB/c mice were the source of the transplant lines used in the present karyological study. Most transplantable plasma cell tumors synthesize and secrete immunoglobulin molecules. Some tumors synthesize them in a balanced manner; *i.e.*, they synthesize and secrete only monomeric (4-chain) γG-like immunoglobulins having a molecular weight of about 150,000, or pentameric γM immunoglobulins or polymeric γA immunoglobulins (*2*). In other tumors immunoglobulin synthesis and secretion are disorderly (*2*). They may synthesize and secrete 1) monomers or polymers and, independently, the specific light-chain subunit; 2) only a specific light chain, and 3) γA halfmers (*2*).

Each transplantable plasma cell tumor is like a clone of protein-producing cells in that each tumor cell synthesizes only one specific type of light and heavy chain. Further, in a transplant

[1] Received April 17, 1968; accepted June 24, 1968.

[2] Supported in part by Public Health Service research grant CA 07798-03 from the National Cancer Institute, grant-in-aid No. 94001 for fundamental scientific research from the Ministry of Education, Japan, and contribution No. 675 from the National Institute of Genetics, Misima, Japan.

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318-443—68——9

line the cells appear to be stabilized, for despite sustained growth, only one type of immunoglobulin molecule is produced.

The primary mineral-oil-induced plasma cell neoplasm arises on the peritoneal surfaces in nodules of oil granuloma. From some early primary tumors more than one type of immunoglobulin-producing transplant line can be established. Chemical and physical analyses of the proteins produced by sister transplant lines derived from the same primary host indicated the sister lines were closely related and could not be regarded as different autochthonous neoplasms (*2*). The four pairs of sister lines examined differed from each other in several ways. One line (MOPC-47A) produced a γA halfmer, while its sister (MOPC-47B) only the specific light-chain subunit of that line.

In another type (Adj PC-6), line (Adj PC-6A) produced a polymeric (serum) γA, while the sister line (Adj PC-6C) had a γA halfmer that appeared in both serum and urine. Tryptic peptide maps of these two proteins differed only by one peptide. In a third type (MOPC-31), one line (MOPC-31C) produced a 4-chain γG-related molecule, and the sister line (MOPC-31B) the same molecule plus an excess of the specific light chain.

In the present investigation, karyological studies of several plasma cell tumors, including sister lines, were made to determine if these lines were related. Chromosome numbers of all lines were abnormal.

MATERIALS AND METHODS

These studies began while Dr. Yosida was a guest worker at the National Cancer Institute, and continued at the National Institute of Genetics, Misima, with the tumor lines sent from Bethesda. This accounts for the karyotypes of different transfer generations of the same tumor (*see* table 1). Chromosome preparations were made on both solid and ascites forms. Chromosomes from ascites tumor cells were easier to prepare, and in a number of studies the solid tumors were converted to the ascites form.

Plasma Cell Tumors

Adj PC-6.—This tumor was induced in 1959 by the intraperitoneal injection of Lieberman's staphylococcal adjuvant (*3*). The original tumor was transplanted into sister lines A and C, two of which are represented in the present study.

The 6A line produced a 7, 9, 11, and 13S γA serum polymer immunoglobulin, which has euglobulin properties (*3*). No γA urinary protein was found in the 6A line. The 6C line produced a γA halfmer (*2*) that appeared in both the serum and urine. The serum protein, however, did not produce 9, 11, or 13S peaks in the ultracentrifuge runs. Recent serological and chemical (*4*) studies showed that these two proteins share important similarities. Notably, each has the same kappa-type light-chain subunit, and each has a common idiotypic antigenic specificity (*4*). The Adj PC-6A line was converted to the ascites form, and a subline of the tumor (Adj PC-6A negative) no longer produced the 6A protein.

Tumors Adj PC-9 (*5*, *6*), RPC-20 (*5*, *6*), and MOPC-88 are represented by only a single line. The MOPC-88 tumors were studied in the primary host.

MOPC-31.—MOPC-31 was induced by mineral oil. Two different protein-producing sister lines were obtained from the primary host: the MOPC-31C, which produced serum protein and urinary protein, and the 31B line, which produced γF serum protein and a kappa-type urinary protein. The 31B and 31C serum proteins have the same tryptic peptide maps, including a similar kappa-type light-chain subunit that lacks tryptic peptide-4 (*2*, *6*).

Two lines, myeloma protein positive and negative, were also observed.[5] The latter was derived from the former. As the original tumor and transplant generations in these lines are uncertain, they are called positive and negative lines in the present paper.

MOPC-70.—MOPC-70 was induced in 1962 and five lines were initiated from the primary host, MOPC-70A–MOPC-70E. Two lines, 70A and 70E, are represented in the present study. Characterization of the lines has been previously described; they produce essentially the same type of protein (a 7S γF serum protein and an excess of urinary light chain). The two lines differ chiefly in the relative

[5] These lines were kindly sent from Mr. N. E. Roberts of the National Cancer Institute.

TABLE 1.—Chromosome numbers, marker chromosomes, and protein specificity of plasma cell neoplasms developed in BALB/c mice

Tumor line	Passage	Protein specificity	Number of chromosomes (mode)	Modal No. of marker chromosomes*	Number of cells observed
Adj PC-6A	23	γA serum polymer	50-79(77)	1 M	42
Adj PC-6A	65	"	64-135(67)	1 M	20
Adj PC-6A positive	56	"	64-176(68)	1 M	50
Adj PC-6A negative	70	None	53-162(72)	2 M, 2 SM	50
Adj PC-6C	33	γA halfmer	70–97(96)	4 M	20
Adj PC-9	72	Kappa chain	53-148(75)	1 M, 2 SM	50
RPC-20	14	Lambda chain	48-153(79)	1 M	54
MOPC-31B	11	γF serum + excess kappa	53-83(81)	None	50
"	38	"	64-135(77)	None	40
"	61	"	76–81(80)	1 TC, 1 m	50
MOPC-31C	10	γF serum only	42-85(44)	1 M	50
"	48	"	40-90(44)	1 M	50
"	59	"	43-115(44)	1 M	50
MOPC-70A	12	γF serum + excess kappa	37-128(73)	None	50
MOPC-10A	69	"	57-68(66)	None	50
MOPC-10E	59	"	63-70(68)	None	50
MOPC-70A negative	82	None	68-86(77)	2 TC, 1 m	30
MOPC-70E	34	γF serum + excess kappa	25-114(79)	None	50
MOPC-88	Primary	γA halfmer	70-89(79)	?	7
"	66	"	48-69(67)	1 M	53
Positive	?	Lambda chain	67-154(77)	1 M, 1 SM, 1 m	50
Negative	?	None	40-145(75)	1 M, 1 SM, 2 m	70

*M=metacentric; SM=submetacentric; m=minute; TC=long telocentric.

amounts of these two proteins they produce. The 70A lines produce more 7S γF myeloma protein than 70E and also 70E produces more urinary protein than the 70A lines. An MOPC-70A-negative subline was also observed. The complete amino acid sequence of the 70E line has been established (*7*). An early transfer generation subline of the 70A line was established with about ten ascites cells (*2*). These lines were designated 70A, 10A, and 70A, 10E.

Cytological Technique

Colchicine solution (0.05 cc of 0.02%) was injected intraperitoneally. One or two hours later the ascites tumor was withdrawn by a glass capillary and treated with hypotonic hyaluronidase solution (20 T.U.R./cc) for 10 minutes and stained with Acetic Dahlia. Permanent slides were made by a modified procedure of that used by Ford and Hamerton (*8*). The tumor cells were treated with 1% sodium citrate in a glass tube, fixed with Carnoy's 3:1 solution, and then stained by Feulgen technique in the tube. Stained material was suspended again in Carnoy's 3:1 solution, and a drop of the material was placed on a glass slide, dried, and mounted. Part of the material was fixed with Carnoy's 3:1 solution after pretreatment with 1% sodium citrate; one drop of the fixed material was placed on a wet slide kept in 50% ethanol, dried quickly over a flame, and stained with lactic acid orcein. Solid tumors were minced with scissors in balanced physiological salt solution, and then chromosome preparations were made according to the above procedures.

RESULTS

Adj PC-6A

A wide distribution of chromosome numbers ranging from 50–79 was observed in the ascites tumor cells at the 23d transplant generation of this line (table 2). Frequency of cells with 77 chromosomes was highest (6 cells) and that of cells with 67, 68, 74, 76, and 78 chromosomes ranked second (3 cells). As marker chromosomes, median metacentrics (M) were observed in all cells, and minutes (m) were also frequent. Among 42 cells 19 had 1 M marker, 16 had 1 M and 1 m marker, 4 had 2 M, and 1 cell had 3 M markers. The chromosomes in 4 cells only were observed in the solid-type tumor of this line. They had 50, 55, 69, and 75 chromosomes. The markers could not be clearly distinguished in 3 of the cells, but 1 cell had a typical M marker and another cell had 1 m marker. In the 65th transplant generation of this line, a narrower distribution of chromosome numbers from 64–68 was observed. Among 50 cells, 26 showed 67 chromosomes and 12 had 66 chromosomes. The third frequency was found at 68 chromosomes (6 cells). Only 1 cell with 135 chromosomes was observed as 2s range. Most (42 cells) had 1 M marker, and 3 showed 1 M and 1 submetacentric (SM) marker. In the other 4 cells only m markers were found. Perhaps, in the course of transplant generations from the 23d–65th, cells with 67 chromosomes and 1 M marker, which were only rarely observed at the 23d transplant generation, were selected to show the highest frequency in the 65th transplant generation.

Adj PC-6A Positive

Among 50 cells of this tumor line, classified into groups s and 2s, 45 were in the s group. Chromosome numbers in this group varied from 64–75, with the mode and average at 68. A stemline with 68 chromosomes may have been selected during 33 transplant generations from Adj PC-6A to the present subline (fig. 1). Among the 50 cells, 43 had M markers, 2 had 1 M and 1 SM marker each, and 4 cells had 4 M markers each. Only 1 cell had no marker. These investigations indicate that the karyotype in the Adj PC-6A positive line resembles that of the parental Adj PC-6A line (table 2).

Adj PC-6A Negative

Among 50 cells observed, 48 were at s range. Chromosome numbers varying from 53–162 chromosomes were observed. The highest frequency (8 cells of each) was cells with 71, 72, and 73 chromosomes. Cells with 69 and 70 chromosomes were next most frequent. Two M and 2 SM markers were observed in 41 cells (fig. 2). One cell among 50 cells analyzed showed 112 chromosomes, which is considered as 2s range. Another cell had 162 chromosomes. The SM marker was observed later in this line, although it was very rare in the parental and the positive line. The karyotype in this protein-negative subline was remarkably different from that of the parental protein-positive line (table 2).

Adj PC-6C

A wide variation of chromosome number (70–97 chromosomes) was also found in the ascites form of this Adj PC-6C line (table 3). The highest frequency was in 4 cells with 96 chromosomes. A remarkable difference was noticed between 6C and 6A lines, in respect to the distribution of chromosome numbers and the number of marker chromosomes. Chromosome numbers in 6C line were distributed at hypertetraploid range, while those of 6A line were distributed at hypotetraploid range. On the marker chromosomes, 6C had 2–6 markers with the mode at 4, but the 6A lines carried 1–2 markers. Among 4 markers in the 6C line, 2 were large M and the other 2 were considerably smaller SM. No m was found in this line (fig. 3). Karyotypes of 7 cells in solid-type tumor in this line were analyzed. The total number

TABLE 2.—Distribution of chromosome numbers in several hypotetraploid tumor lines

Tumor line	Passage	Chromosome counts																											Number cells observed	Mode	Average chromosome No.		Markers*			
		<50	51–60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	86–90	91<			s-cells	2s-cells	M	SM	TC	m
MOPC-88	66	2	4	1	—	2	2	3	7	17	14	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	53	67	65.2	—	1	—	—	—
Adj PC-6A	23	1	2	1	1	2	1	2	—	3	3	1	1	2	2	1	3	3	3	6	3	1	—	—	—	—	—	—	42	77	70.4	—	1	—	—	—
Adj PC-6A	65	—	—	—	—	—	3	2	12	26	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	50	67	66.6	135.0	1	—	—	—
Adj PC-6A positive	56	—	—	—	—	—	1	—	3	8	25	5	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—	5	50	68	68.0	132.5	1	—	—	—
Adj PC-6A negative	70	—	2	1	1	1	—	1	1	3	1	7	6	8	8	8	—	—	—	—	—	—	—	—	—	—	—	2	50	72	69.3	137.0	2	2	—	—
MOPC-70A	12	5	2	—	1	—	—	—	1	2	1	2	5	11	4	12	—	—	1	—	—	—	—	—	—	—	—	3	50	73	69.9	125.0	—	—	—	—
MOPC-10A	69	—	2	—	—	2	2	5	23	13	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50	66	65.7	—	—	—	—	—
MOPC-10E	59	—	—	—	—	1	—	2	2	6	34	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50	68	67.7	—	—	—	—	—
MOPC-70A negative	82	—	—	—	—	—	—	—	—	—	1	—	—	—	2	—	2	4	7	8	4	1	—	—	—	—	1	—	30	77	76.2	—	—	—	2	1
Adj PC-9	72	—	1	—	—	—	—	—	—	—	—	1	2	—	4	6	10	22	1	—	—	—	—	—	—	—	—	3	50	75	73.5	139.3	1	2	—	—
RPC-20	14	1	—	—	1	—	1	—	—	—	2	—	—	1	—	—	2	4	2	6	10	13	6	2	2	—	—	1	54	79	76.5	153.0	1	—	—	—
Positive	69	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	2	5	15	19	4	—	—	—	—	—	—	3	50	77	76.7	141.0	1	1	—	2
Negative	70	5	—	—	—	—	—	—	—	—	1	2	5	7	8	10	10	16	3	—	—	—	1	—	—	—	—	2	70	75	73.1	143.5	1	1	—	2
MOPC-31B	11	—	2	1	1	1	—	—	—	—	2	1	—	—	1	1	2	2	1	2	4	6	3	18	1	1	—	—	50	81	76.4	—	—	—	—	—
"	38	—	—	—	—	—	1	—	—	—	—	1	1	1	—	1	1	1	8	9	8	7	—	—	—	—	—	1	40	77	76.3	135.0	—	—	—	—
"	61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	1	6	13	27	1	—	—	—	—	50	80	79.3	—	—	—	1	1

*M = metacentrics; SM = submetacentrics; TC = long telocentrics; m = minutes.

TABLE 3.—Distribution of chromosome number in the 33d transplant generation of a hypertetraploid Adj PC-6C line*

Number of chromosomes	70	71–80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	Total
Number of cells observed	1	1	1	—	2	1	—	—	1	2	—	3	—	—	—	1	2	4	1	20

*Average chromosome number was 88.8. Four metacentric markers were observed in most cells.

of chromosomes and M and SM markers is given as follows:

Total chromosomes	Number of markers	Number of cells
42	4	1
63	3	2
66	4	2
67	4	1
67	?	1

The total number of chromosomes in the solid-type tumor was significantly lower than in the ascites form, but the number of M and SM markers was about the same in both types.

Adj PC-9

Chromosomes in 2 cells only were observed in the ascites form of this line at the fourteenth transplant generation. One had 77 chromosomes, including 1 rather small SM element (fig. 4), and the other had 80 chromosomes with the same marker. At the 72d transplant generation, 50 cells were again observed. The chromosome number was distributed from 53–76 in s-range cells, among which those with 75 chromosomes were at highest frequency (22 cells, fig. 5). Three marker chromosomes, 1 M and 2 SM (1 large and 1 small) were seen in 44 of 50 cells analyzed (table 2).

RPC-20

A wide variation in chromosome number (48–153) was found among 54 cells of the ascites form at the fourteenth transplant generation of this line (table 2). Cells with 79 chromosomes had highest frequency (13 cells), those with 78 chromosomes, second (10 cells), and those with 77 and 80 chromosomes, third (6 cells). Among 54 cells, 29 had 1 small M chromosome. No marker was found in 8 cells, while 7 cells had 2 M markers; the remaining few cells had 3 or 4 M and SM markers. Minutes were also observed in a few cells. From this investigation, cells with 77–80 chromosomes including 1 M marker seem to represent the stemline karyotype of this line.

MOPC-31B

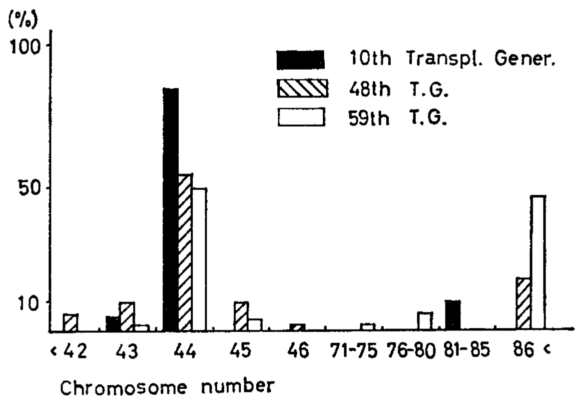
Chromosome numbers of 50 cells in the ascites line of the eleventh transplant generation ranged from 53–83, showing the highest occurrence in cells with 81 chromosomes (18 cells). Cells with 79 chromosomes ranked second in frequency (6 cells, table 2). About half (23 cells) the observed cells had no markers (fig. 7), but 16 cells had 1 M marker (fig. 8) and the remaining 11 cells had 2–4 M and SM markers. From this investigation, it is assumed that cells with 81 chromosomes without markers represent the stemline in the ascites form of this tumor line.

In the 38th transplant generation the distribution of chromosome number is like that in the eleventh generation, although the reduction of a few chromosomes was found in the cell population of this generation. The cells with 76–78 chromosomes were observed most frequently. Average chromosome number of 39 cells in the s range was 76.3. Most cells had no marker chromosome, but an extra-long TC element (L marker) was found in many cells.

In the 61st transplant generation about 90% of the cells had the long TC marker with 1 M (fig. 8). Eight percent of the cells had the m marker in addition to the M and long TC markers. The remaining 2% had only m marker.

MOPC-31C

In the tenth transplant generation, karyotypes of 50 tumor cells were analyzed first. The chromosome number was distributed from 42–85, with the mode at 44 (37 cells) (fig. 11). Among 50 cells, 94% had 1 M marker. Two cells among them were at the 2s level. In the 48th transplant generation, karyotypes in 50 metaphase cells were analyzed a second time. Among them, 41 cells had hyperdiploid chromosome numbers distributed from 40–46, with the mode at 44 chromosomes (27 cells), while 9 cells were at hypertetraploid level (fig. 9). About 90% of the cells had 1 M marker, and 1 cell had no marker. Two M markers were observed in most hypertetraploid cells. A remarkable increase of cells at the 2s level was observed in the 59th transplant generation. Among 50 cells, 28 were at the s level, while 22 were at the 2s level. Twenty-five among twenty-eight cells at the s level were characterized by the stemline karyotype with 44 chromosomes, including 1 M marker. Among 22 cells at the 2s level, 8 had 88 chromosomes, which is double the chromosome set of the stemline cells; 17 cells had 2 M markers. The change of chromosome number in the 3 different transplant generations is shown in text-figure 1. As seen in the text-figure, the frequency of cells at the 2s level increased progressively during transplantations.



TEXT-FIGURE 1.—Change of frequencies of hyperdiploid and hypertetraploid cells according to the progress of transplant generations of MOPC-31C. The frequency of cells with hyperdiploid (about 44 chromosomes) decreased in the progress of transplant generations, while that in hypertetraploid cells increased.

MOPC-70A

Chromosomes of 50 cells in ascites form of this line were observed. Chromosome number varied from 37–128. Among them the highest frequency (12 cells) was that of cells with 73 chromosomes. Frequency of cells with 71 chromosomes ranked second (11 cells). No markers were found in most cells (46 cells). This investigation shows that the stemline of the ascites tumor was characterized by cells with 70–73 TC chromosomes.

MOPC-70A and MOPC-10A

Narrower distribution of chromosome numbers from 57–68, with mode at 66, was found in these lines (fig. 10). Among 50 cells analyzed, 23 had 66 chromosomes. No marker chromosome was found in 46 cells (92%), but 2 cells had 1 m each and the remaining 2 had 1 SM element each (table 2).

MOPC-70A and MOPC-10E

Chromosome numbers of 30 cells were distributed from 63–70, showing the highest frequency in the cells with 68 chromosomes (73%). Marker chromosomes were not found in 90% of the cells (table 2). An m marker was observed in only 3 cells.

MOPC-70A Negative

In this line, chromosome numbers were distributed from 68–86 in 30 cells. Among them 8 cells showed 77 chromosomes and 7 cells had 76 chromosomes. All cells had some extra-long TC chromosomes in their karyotypes as well as 1 or 2 m markers. About half the cells had 2 extra-long TC and 1 m marker (table 2).

MOPC-70E

Distribution of chromosome numbers in 50 cells of this line is shown in table 4. Chromosome number was distributed in hypotriploid range, showing the highest frequency in the cells with 57 chromosomes. Cells with 58 chromosomes ranked second in frequency (10 cells), and cells with 56 chromosomes ranked third (7 cells). Average chromosome number in the hypotriploid cells was 56.0. No marker chromosomes were observed in all 50 cells (fig. 12).

MOPC-88

The primary plasma cell neoplasm in this line had hypotetraploid chromosome constitution. Dis-

TABLE 4.—Distribution of chromosome numbers in the 34th transplant generation of a hypotriploid MOPC-70E line

Number of chromosomes	<40	41–45	46–50	51	52	53	54	55	56	57	58	59	114	Total
Number of cells observed	2	2	2	—	1	—	1	2	7	17	10	5	1	50

tribution of near-diploid and near-tetraploid cells was examined in 118 metaphase cells. Among them, 108 cells had near-tetraploid karyotypes, 16 showed near-diploid, and one cell had near-triploid constitution. From this result the plasma cell neoplasm had near-tetraploid karyotypes in the primary stage of development. Exact chromosome numbers and karyotypes were examined in 7 cells. They had 70, 73, 79 (3 cells), 83, and 89 chromosomes. All chromosomes were characterized by telocentric centromeres.

In the 66th transplant generation, chromosomes of 53 metaphase cells were observed (table 2), and a considerably wider distribution of chromosome numbers from 48–69 was noted. Among 53 cells, 17 had 67 chromosomes and 14 showed 58 chromosomes. Cells with 66 chromosomes ranked third (7 cells). Average chromosome number of 53 cells was 65.2. Among 53 analyzed cells, 44 had 1 M marker. No marker was found in 5 cells and two M markers were found in the remaining 4 cells.

Protein-Positive Line

Chromosomes of 50 cells of this line were analyzed. Among them, 47 cells were hypotetraploidy (s) and 3 were in the 2s range. Among 47 cells, 19 showed 77 chromosomes and 15 showed 76. One SM, one M, and one m marker were observed in 37 of 50 cells (fig. 6). About 90% of the cells had 1 m marker, although the m was observed in only a few cells at the fourteenth transplant generation of parental RPC-20 line. Two TC chromosomes with secondary constriction near the centromere (fig. 6) were usually observed in this line.

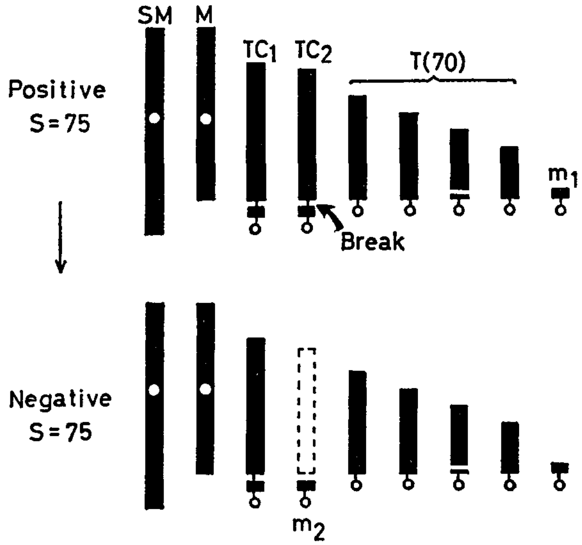
Protein-Negative Line

Karyotypes of the nonproducing protein-negative line were analyzed in 70 metaphase cells (table 2). Cells with 75 chromosomes were observed most often (16 cells), and cells with 73 and 74 chromosomes ranked second (10 cells in each). Average chromosome number in 63 hypotetraploid cells was 73.1. Among 70 cells, 28 had 1 M, 1 SM, and 2 m markers, and the other 10 cells had the same markers, but 3 m's were included in their karyotypes. Other cells showed various combinations of these 3 markers, as seen in the table. A comparison between karyotypes of positive and negative lines indicates that 2 TC elements in stemline cells of the positive line were lost in those of the negative line, and 1 m was newly added in the latter line. Only 1 TC chromosome was observed in the negative-line karyotype, although 2 were usually observed in the positive-line karyotype.

These investigations suggest that the negative-line karyotype arose from cells with 75 chromosomes in the positive line, according to the following hypothetical scheme: 1) breakage at the secondary constriction of a TC chromosome, included in the positive-line karyotype; 2) a small chromosome with centromere, which resulted from the breakage, remaining in the negative-line karyotype as a new m; 3) loss of an acentric long fragment from the new karyotype. The karyotypic change in stemline cells of both positive and negative lines is shown in text-figure 2.

DISCUSSION

Several findings in the present study relating to karyotypes are summarized as follows: Most neoplasms observed were characterized by hypotetraploid chromosome numbers, but one line was hyperdiploid (MOPC-31C), one line was hypotriploid (MOPC-70E), and one was hypertetraploid (Adj PC-6C). In the earlier transfer generations the chromosome number was wider; later the chromosome number tended to cluster around a mode. Many tumors contained as many as 1–4 marker chromosomes (M, SM, TC, and m). Marker chromosomes in the tumor cells seemed alike in size and shape. For instance, M markers observed in Adj PC-6A (fig. 1), 6C (fig. 3), Adj PC-9 (fig. 5), RPC-20 (fig. 6), MOPC-31B (fig. 8),



TEXT-FIGURE 2.—Diagram of chromosome alteration from a karyotype of plasma cell tumor positive line to that of negative line. SM = submetacentric, M = metacentric, TC = telocentric with secondary constriction, and m = minute markers. Chromosome breakage occurs at the constriction of a TC chromosome in the positive line, and the short arm with a centromere remains as a new m in the negative line. Long arm without centromere is lost (*broken line* in the negative line).

MOPC-31C (figs. 9 and 11) are very similar. Large SM chromosomes were observed in Adj PC-6A negative (fig. 2) and RPC-20 (fig. 6), and small SM elements found in Adj PC-6C (fig. 3) and Adj PC-9 (figs. 4 and 5) were also alike in shape. Similar m's were observed in several tumor lines. Although there was a similarity in shape of marker chromosomes, a characteristic marker chromosome relating all the plasma cell tumors was not found. Abnormality in chromosome number appeared in the early transfer generations.

One neoplasm observed in the primary host (MOPC-88) was aneuploid. The large size of both primary and transplantable neoplastic plasma cells (*9*) correlated with the near-tetraploid chromosome number, suggesting that abnormality in chromosome number may be established very early in the origin of the neoplastic plasma cell.

The chromosome numbers and karyotypes did not establish any specific relationship to immunoglobulin formation. In the mouse, 4 heavy-chain genes are closely linked in a single locus (*10, 11*), but nothing is known about the loci controlling light chains. Possibly the mouse resembles the rabbit in that the light chain loci are dissociated from the heavy chain gene loci. If so, some abnormalities in chromosome number could produce gene dose effects. Interestingly, the 2 MOPC-31 lines differed: The 31C line that produces a balanced synthesis of monomeric 4-chain γF immunoglobulin possessed a near-diploid number, while the near-tetraploid 31B line that produces the same γF myeloma protein had also an excess of light chain.

Fjelde *et al.* (*12*) examined 4 plasma cell leukemias in mice; 1 was hypotriploid and the other 3 were near-diploid. The plasma cell leukemias differ from the plasmacytomas chiefly in that they are composed of undifferentiated reticular cells rather than plasma cells. Immunoglobulin production by these tumors is relatively transient and is soon lost during transplantation.

Chromosomes of a plasma cell neoplasm X5563, developed in a C3H mouse, were observed by Yosida *et al.* (*13*). In this case near-tetraploid chromosomes were also observed in solid and ascites tumors. This means that tetraploid karyotypes in plasma cell neoplasms are not specifically those of BALB/c mice only.

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PLATES

FIGURE 1.—Metaphase chromosomes in a stemline cell of mouse plasma cell tumor Adj PC-6A positive (protein-producing) line. Chromosome number is 68. *Arrow* indicates metacentric marker.

FIGURE 2.—Metaphase chromosomes in a tumor cell of Adj PC-6A negative (protein-nonproducing) line; 70 chromosomes are counted. *Arrows* indicate 4 metacentric marker chromosomes.

FIGURE 3.—Metaphase chromosomes in a tumor cell of Adj PC-6C line; 88 chromosomes are included. *Arrows* indicate 4 markers.

FIGURE 4.—Metaphase chromosomes in a tumor cell at the fourteenth transplant generation of Adj PC-9 line; 77 chromosomes including 1 small submedian metacentric marker (*arrow*) are visible.

FIGURE 5.—Tumor cell at the 72d transplant generation of Adj PC-9 line; 75 chromosomes including 3 markers are counted. *Arrows* indicate markers.

FIGURE 6.—Chromosomes of a tumor cell in protein-producing (positive) line; 77 chromosomes are counted. Among them 1 submetacentric (SM), 1 metacentric (M), 1 minute (m), and two telocentric (TC) chromosomes with secondary constriction near terminal centromere were observed.

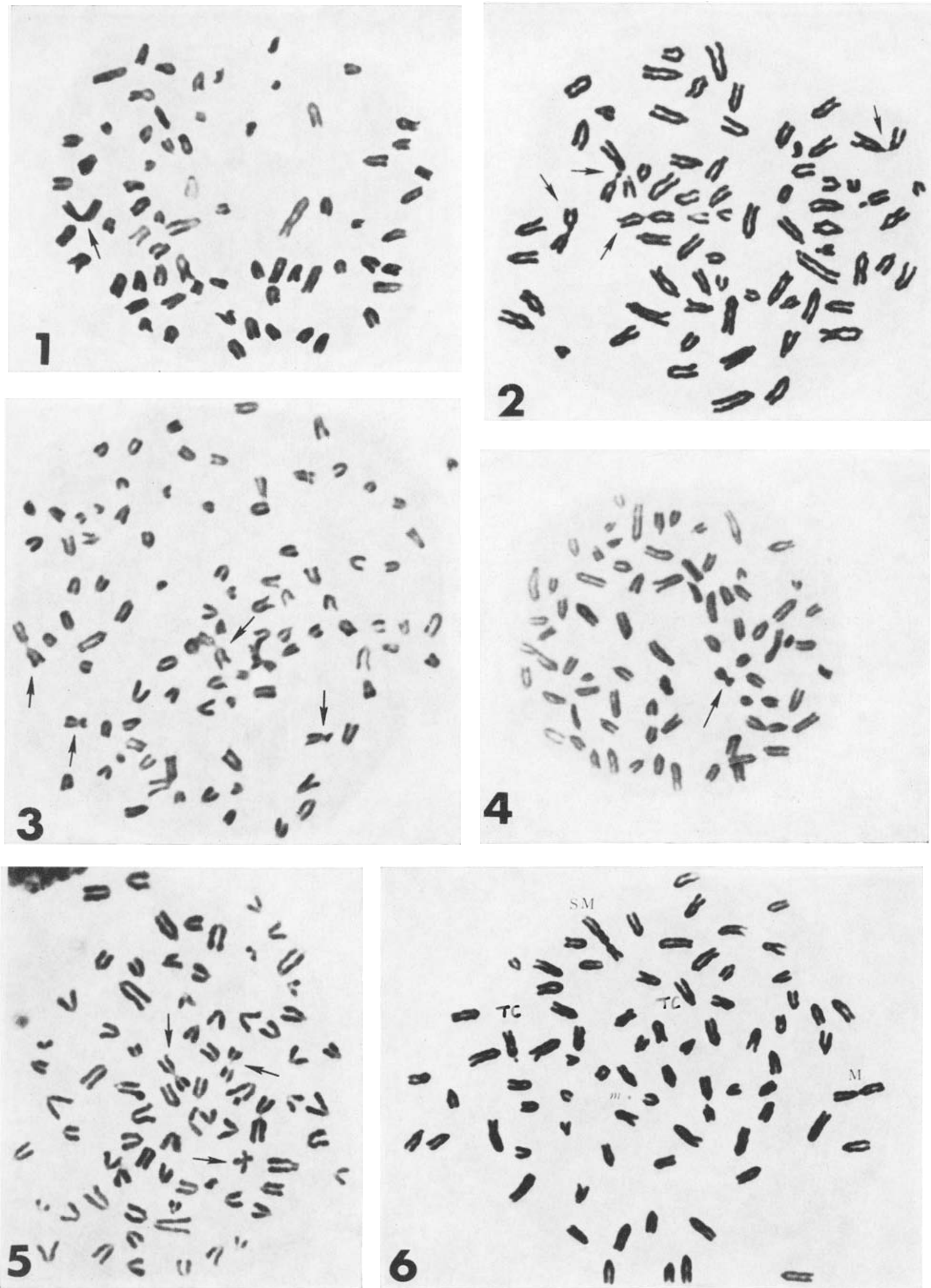
1
2
3
4
5
6
SM
TC
TC
M
m

FIGURE 7.—Chromosomes of a tumor cell at the eleventh transplant generation in MOPC-31B line; 79 chromosomes without any markers are denoted.

FIGURE 8.—Chromosomes of a tumor cell in the 61st transplant generation of MOPC-31B line. One extra-long element (L), 1 minute (m), and 1 metacentric (M) marker are denoted.

FIGURE 9.—Chromosomes of a tumor cell at the 59th transplant generation of MOPC-31C line; 89 chromosomes include 2 M markers.

FIGURE 10.—Chromosomes of a tumor cell in MOPC-70A and MOPC-10A lines; 66 chromosomes without markers are shown.

FIGURE 11.—Chromosomes of a tumor cell at the 48th transplant generation of MOPC-31C line; 44 chromosomes including 1 M marker are denoted.

FIGURE 12.—Chromosomes of a tumor cell in MOPC-70E line; 57 chromosomes without any marker are shown.

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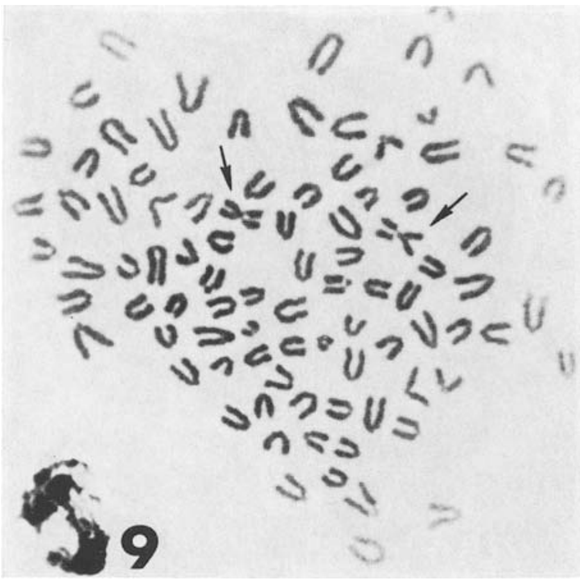
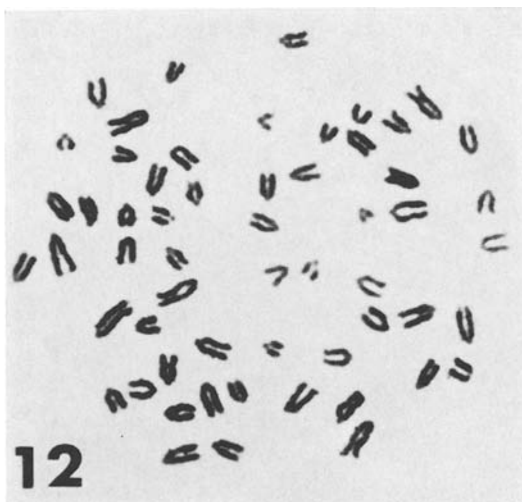


9

10

11



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