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Received March 31, 1964. P.S.E.B.M., 1964, v116.

The Chromosomes of Tumors Induced in Mice by Human Adenovirus, Type 12.* (29464)

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Human adenovirus, type 12, when injected into newborn hamsters(1), rats(2), mice(3), or mastomys(4) induces undifferentiated tumors at site of injection. This communication concerns a chromosome study of tumors so induced in mice.

Materials and methods. Cells were studied from primary tumors in four C_3HfGs mice and from 71 tumors representing the first (37 animals) and second (34 animals) passages of these tumors. The 4 primary tumors were obtained from animals (2 females, 2 males) injected intraperitoneally when newborn with 0.05 ml of human adenovirus type 12 ($A_{12}S_9$). Most of the animals used for tumor passage were males and were injected with tumor mince or cell suspensions when adult. The resulting tumors were harvested after 2-6 weeks of growth. Two of the animals with primary tumors were treated with colchicine and 2 were untreated. All of the animals carrying tumor passages were colchicine treated (1 μg per gram body weight). The animals were injected with colchicine 1-3 hours before they were sacrificed by rupture of the cervical spinal cord. Tissue culture studies were done on all animals, and sections for pathology on most. Routine air dry techniques and temporary squash methods were used in making the chromosome preparations from finely minced tumor tissue. Most of the studies for structural aberrations were done from temporary orcein squash preparations, since these had a higher proportion of cells with chromosomes lying separately in one plane. The minced tissue was suspended in saline, which was subsequently diluted 1:4 with distilled water, allowed to stand 7-20 minutes, and then centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted, and 1 ml of fixative (60% acetic acid) was layered over the button of cells. After one hour or longer the acid was decanted, and 1 ml of acid orcein dye (2% orcein in 60% acetic acid) was added. The cells, resuspended in the solution, were used for making squash preparations. Terminology for chromosome variation is employed in accordance with definitions used in classical genetics adapted to present day cytogenetics(5).

Results and discussion. Results of the investigation are summarized in Tables I and II. Material from the original tumors, passage tumors, and tissue cultures showed essentially the same findings: aneuploidy, polyploidy, mixoploidy, low rates of endoploidy (5/2001 cells), and relatively few structural aberrations (17/804). Very few chromosomes other than normal telocentrics were present. Most of the cells (2001) analyzed for chromosome number (Table I) were in the diploid range of 30 to 50, 56% having the normal number of 40 telocentric chromosomes per cell. Fig. 1, showing the distribution of the chromosome number in the original tumors and the first and second passages of the tumors, demonstrates the likely presence of mixoploidy. All show a peak at 42 ($2N = S = 2x + 2 = 42$) and 55 ($2N = S = 2x + 15 = 55$) as well as peaks at 3x (excepting original tumors), 4x, 5x and higher. There were no marker chromosomes present, or any

* Supported by grants from the Anna Fuller Fund and Nat. Cancer Inst., U.S.P.H.S.

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consistent finding which could characterize the tumors, other than a slight tendency toward variation in size of the smallest and largest chromosomes. Only the very smallest were called minute chromosomes and considered aberrations (Table II, Fig. 2). The tu-

TABLE I. Distribution of Chromosomes in Metaphase Cells of Human Adeno 12 Virus Tumors in Mice.

Source:	Virus treated mice	1st & 2nd tumor passage	Combined results
No. mice:	4	71	75
Total No. cells:	302	1699	2001
Hypodiploid cells	24	156	180
Diploid (40)	193	934	1127
Hyperdiploid	29	272	301
Total	246	1362	1608
Range	30–49	31–50	30–50
%	81.5	80.2	80.4
Hypotriploid cells	11	86	97
Triploid (60)	0	26	26
Hypertriploid	4	17	21
Total	15	129	144
Range	52–62	52–69	52–69
%	5.0	7.6	7.2
Hypotetraploid cells	9	38	47
Tetraploid (80)	16	122	138
Hypertetraploid	4	10	14
Total	29	170	199
Range	78–83	75–84	75–84
%	9.6	10.0	9.9
Pentaploid and higher cells	12	38	50
Range	100–250	100–250	100–250
%	4.0	2.2	2.5
Endopolyploidy	1/302	4/1699	5/2001
Mixoploidy	+	+	+

TABLE II. Aberrations Seen in Murine Tumors Induced by Human Adeno 12 Virus.

Source	No. cells with aberrations	%	Type of aberrations	Chromosome No.
I: Virus treated mice	4/304	1.3	1. Break, ring	62
			2. Metacentrics, break, rejoining	142
			3. Metacentrics, breaks, constrictions, an extra long chromosome	101
			4. Fragmentation, minute chromosomes	42
II: 1st & 2nd passage	13/500	2.6	1. Ring—break	250
			2. Breaks	42
			3. "	78
			4. "	80
			5. Metacentric and extra long No. one	42
			6. Breaks and extra long No. one	42
			7. Break	49
			8. Breaks and 2 extra long No. one	76
			9. Minute chromosome	100
			10. Metacentric, constriction	43
			11. Metacentric, long No. one with breaks	55
			12. Fragmentation, endoreduplication, minute chromosome	100
			13. Fragmentation, minute chromosome	250
Totals	17/804	2.1		

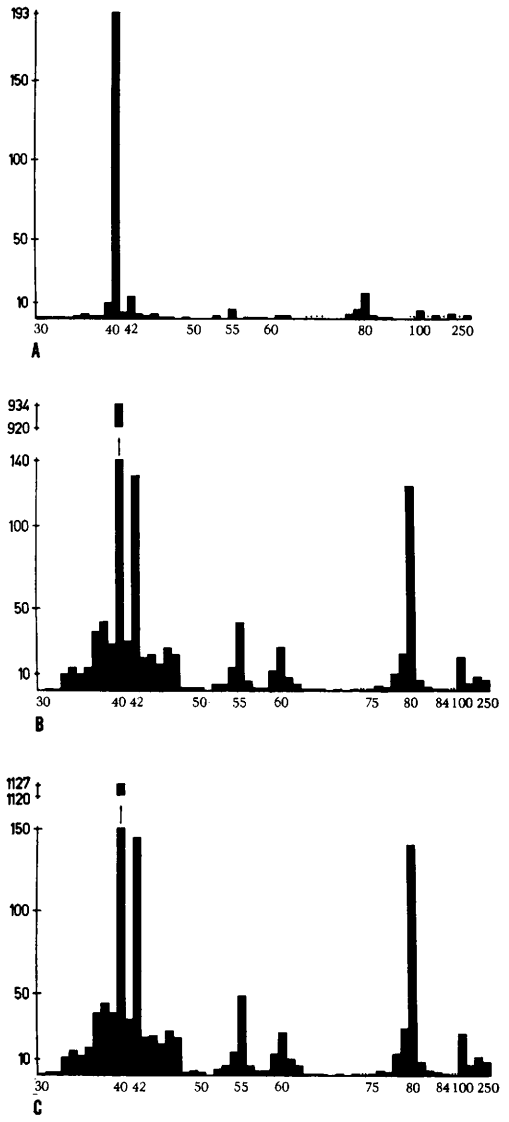

FIG. 1. Graphs, showing modality peaks at 42 and 55 (suggesting mixoploidy) and presence of the normal diploid stemline (2 N) at 40, and multiples of (N) at 3×, 4×, 5×, and higher. (A) represents cells from the 4 original virus treated animals, (B) represents cells from 71 animals with 1st and 2nd tumor passages. (C) shows combined results of (A) and (B).

mor cells seemed to be fragile, and it is likely that the high number of cells with less than 40 chromosomes is the result of technical difficulties rather than an actuality. The tumors appear not to have reached a stabilized karyotype or stemline, but show the findings one would expect in a shifting cell population. The findings are consistent with studies reported in the literature(6).

The percentage of structural aberrations (Table II, Fig. 2, 3) was relatively low as compared to some virus-induced chromosome aberrations that have been reported(7,8,9, 10). However, it is possible that changes occurred on a molecular level (gene mutations), rather than on the chromosome level, reflected by structural variation. Numerical changes and ploidy changes, however, were observed, suggesting rearrangements on the whole chromosome level. It is therefore pos-

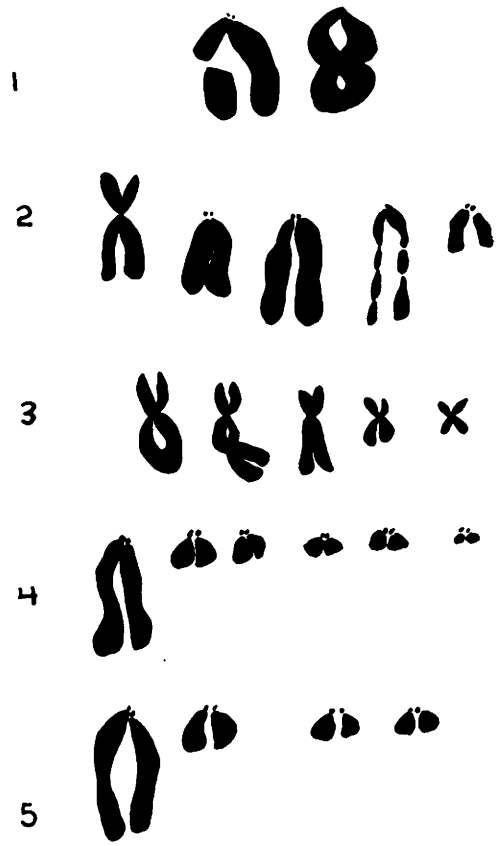

FIG. 2. Types of abnormal chromosomes seen in primary tumors induced in mice by human adenovirus, type 12, from cells containing 62 (1), 101 (2), 142 (3), 42 (4) and 42 (5) chromosomes respectively. From left to right: (1) A chromatid break and a ring chromosome. (2) A metacentric, a normal No. 1 chromosome, an extra long No. 1 chromosome, an extra long No. 1 chromosome wtih constrictions, a chromatid break. (3) Five metacentric chromosomes, one (left) showing fused arms. (4) Variation in size of smallest chromosomes. Left, normal No. 1; extreme right, minute chromosome. (5) Normal No. 1 and 3 small chromosomes.

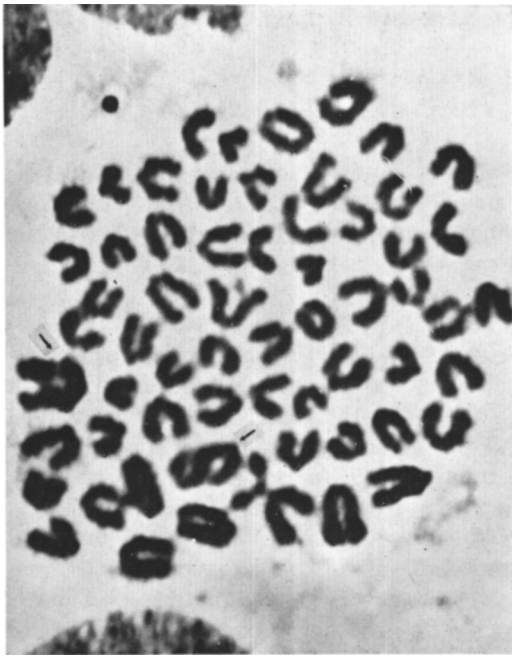

FIG. 3. A metaphase plate showing a cell with a ring chromosome and a chromatid break (arrows) (No. 1, Fig. 2).

sible that aberrations at the whole chromosome level took place early in the stage of tumor development and were not detectable at the time the tumors were analyzed. Indeed, Stitch *et al* (11) have observed that human adenovirus type 12 induced a high incidence of chromosome aberrations in a tissue-cultured chinese hamster diploid cell strain, but that most of the aberrations were lethal and did not survive subculturing. It is possible that one of the few surviving virus-induced chromosome aberrations, whether visible or invisible, is responsible for malignant transformation. It is now known that although complete infectious adenovirus-12 cannot be recovered from the tumors induced by it, a type specific complement-fixing adenovirus-12 antigen does continue to replicate in the primary, transplanted, and tissue cultured cells of tumors induced by it in the hamster(2), the rat(2) and the mouse(12). It is thus possible, if not probable, that the significant aberration is not a chromosome breakage, deletion or translocation, but rather the introduction of a portion of the viral genome.

Summary. A chromosome study was done on 4 primary tumors and 71 passage tumors induced in mice by human adenovirus type 12. Fifty-six percent of the cells analyzed (1127/2001) showed the normal stemline (40 telocentrics), and 80% of the cells were in the diploid range (30 to 50). Aneuploidy, polyploidy, mixoploidy, low rates of endoploidy (5/2001), and relatively few structural aberrations (17/804) were present. Structural aberrations consisted of metacentrics, breaks, rejoining constrictions, rings, and extra long and minute chromosomes. Modality peaks at 42 and 55 were present; 7.2% of the cells were in the triploid range (52 to 69), 9.9% in the tetraploid range (75 to 84), and 2.5% were pentaploid and higher.

The authors wish to thank Professor Albert Levan for comments and discussion prior to publication, and Drs. L. Samper, K. R. Utsumi and G. L. Van Hoosier, Jr., for technical assistance.

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Received April 6, 1964. P.S.E.B.M., 1964, v116.