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Identification of Aneuploidy-Inducing Agents Using Cytokinesis-Blocked Human Lymphocytes and an Antikinetochore Antibody

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The identification of agents causing aneuploidy in humans, a condition associated with carcinogenesis and birth defects, is currently limited due to the highly skilled and time-consuming nature of cytogenetic analyses. We report the development of a new simple and rapid assay to identify aneuploidy-inducing agents (aneuplaidogens). The assay involves the chemical- or radiation-induced formation of micronuclei in cytokinesis-blocked human lymphocytes and the use of an antikinetcchare antibody to determine whether the micronuclei contain centromeres — condition indicating a high potential for aneuploidy. All agents tested produced dose-related increases in the frequency of micronucleated cells. The micronucleated cells induced by the known oneuploidogens — colchicine, vincristine sulfate, and diethylstilbestrol — contained kinetochore-positive micronuclei 92, 87, and 76% of the time, respectively. In contrast, the micronucleated cells induced by the potent clastogens — ionizing radiation and sodium arsenite – contained kinetochore-positive micronuclei only 3 and 19% of the time, respectively. These results indicate that this relatively simple assay can discriminate between aneuploidogens and clostogens ond may allow a more rapid identification of environmental and therapeutic agents with aneuploidy-inducing patentiol.

Key words: micronucleus assay, colchicine, vincristine sulfate, diethylstilbestrol, sodium arsenite, ionizing radiation

INTRODUCTION

Aneuploidy, a condition in which the chromosome number of a cell or individual differs from a multiple of the haploid state, is associated with spontaneous abortions, congenital malformations, mental retardation, and carcinogenesis [Sandberg, 1980; Hecht and Hecht, 1987]. Associations between aneuploidy and neoplastic development have been observed in studies of humans with congenital and familial predispositions for cancer [Cavenee et al., 1983; Evans, 1985], as well as in studies of patients and workers with acute nonlymphocytic leukemia resulting from chemotherapy and benzene exposure [Erdogan and Aksoy, 1973; Rowley, 1983; Pedersen-Bjergaard and Philip. 1987]. Similar results are observed in animal and cellular systems in which the nonrandom gain or loss of certain chromosomes is associated with chemical, radiation, viral. or spontaneous induction of tumors or neoplastic transformation [Sasaki, 1982; Miller and Miller, 1983; Knuutila. 1987].

Although in many tumor cells these changes appear to be a secondary effect related to rapid cell proliferation [Evans, 1985; Rubin, 1985], a growing body of molecular and cytogenetic evidence indicates that the induction of aneuploidy may play an important and possibly essential role in the neoplastic development of certain tumors [Yunis. 1983; Cavenee et al., 1983; Oshimura and Barrett, 1986; Conti et al., 1986; Oshimura et al., 1988].

A significant number of human carcinogens, such as benzene, diethylstilbestrol, ethanol, and asbestos, which have been reported as nonmutagenic in standard mutation assays. have been shown *to* interfere with chromosome segregation during mitosis and *to* result in aneuploidy [Danford, 1985; Barrett et al., 1987; Waters et al., 1988]. In spite of evidence for the involvement of aneuploidy and aneuploidogens (aneuploidy-inducing agents) in the carcinogenic pro-

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cess, rapid assays for identifying these agents using systems relevant to humans are not well developed [Dellarco et al., 1986; Galloway and Ivett, 1986; Oshimura and Barrett, 1986]. The standard procedure for determining aneuploidogens in mammalian systems involves counting individual chromosomes in metaphase plates and identifying the frequency of cells that differ from the diploid or normal number. This method is time consuming, requires highly skilled personnel, and is prone to technical artifacts resulting in artificially high rates of hypodiploidy [Brown et al., 1983; Oshimura and Barrett, 19861. In vitro assays with nonmammalian systems have been developed but have limited value for human risk estimation in that considerable phylogenetic differences exist between species both in the metabolism and in the mechanism and kinetics of cell division [Parry and Parry, 19871.

Some investigators have proposed using micronuclei induction as an assay for aneuploidy [Parry and Parry, 1987; Oshimura and Barrett, 19861. A major limitation in this approach has been that micronuclei can arise from entire chromosomes or from chromosomal fragments. and therefore the assay fails to discriminate between aneuploidogens and clastogens. Numerous studies to distinguish between chromosome- and fragment-containing micronuclei based upon DNA content [Heddle and Carrano, 1977], size [Yamamoto and Kikuchi, 1980; Valadaud-Banieu, 1983; Hogstedt and Karlsson, 1985], hybridization experiments [Viaggi et al., 1987], and centromeric heterochromatin [Banduhn and Obe, 1985] have been attempted. Recently, a number of investigators [Brinkley et al., 1985; Frackowiak et al., 1986; Vig and Swearngin, 1986] have reported using an antikinetochore antibody isolated from the serum of scleroderma patients to demonstrate the presence of kinetochores in micronuclei. We have combined this approach with the cytokinesis-blocked lymphocyte method developed by Fenech and Morley [1985] to develop a relatively rapid assay for aneuploidy induction using human cells. The scoring of micronuclei in cytokinesis-blocked (i.e., binucleated) cells has several advantages that are invaluable in developing an assay for aneuploidy. Binucleated cells are the result of a single mitosis and are scored during interphase so artifactual loss of chromosomes is not a problem. In addition, scoring is rapid and simple, requiring minimal training and experience, and staining is fast and reliable. This assay is based upon the assumption that a micronucleus containing a kinetochore (and therefore presumably the centromere and entire chromosome) in a dividing micronucleated cell will segregate with only one of the daughter cells. Cytochalasin B is used to block cytokinesis and facilitate scoring. However, had the micronucleated cell been allowed to divide, the resulting micronucleated and nonmicronucleated daughter cells both have a high probability of being aneuploid. We now report that the modified micronucleus test using an antikinetochore antibody has a potential application as an assay for aneuploidy-inducing agents. The method is capable of distinguishing between the aneuploidogens colchicine, vincristine sulfate, and diethylstilbestrol (DES) [Oshimura and Barrett, 19861, and the clastogens sodium arsenite [Wan et al., 19821 and ionizing radiation [Heddle and Carrano, 19771.

MATERIALS AND METHODS

Chemicals and Media

Vincristine sulfate, diethylstilbestrol, and sodium arsenite were purchased from Aldrich (Milwaukee, WI). Colchicine, polyoxyethylenesorbitan monolaurate (Tween 20). 4′-6-diamidino-2-phenylindole (DAPI), *p*-phenylenediamine dihydrochloride, gentamycin sulfate, and cytochalasin B were obtained from Sigma (St. Louis, MO). The antikinetochore antibody (centromere-positive control serum for the antinuclear antibody test) and the fluorescent rabbit anti-human gamma globulin were purchased from Antibodies Incorporated (Davis, CA). Minimum essential medium alpha, Dulbecco's phosphate-buffered saline (PBS), L-glutamine, and phytohemagglutinin were purchased from Gibco (Grand Island, NY). Fetal bovine serum was purchased from Hyclone (Logan, UT) and sodium heparin was purchased from Invenex (Chagrin Falls, OH).

Culture Media and Conditions

Blood was obtained from a healthy male volunteer by venipuncture using heparinized vacutainers and was mixed thoroughly. Lymphocytes were isolated using LeucoPREP cell separation tubes (Becton Dickinson, Lincoln Park, NJ) and were cultured in the dark for 72 hr under a 5% CO_2 atmosphere at an initial density of 0.5 x 10^6 cells/ml. The culture medium consisted of minimum essential medium alpha. 16% fetal bovine serum, gentamycin sulfate (final concentration 0.048 mg/ml), L-glutamine (final concentration 2 mM), 1% sodium heparin, and 2.36% phytohemagglutinin (M form). Radiation treatment was performed at 24 hr using a ^{137}Cs source at a dose rate of 33.3 rad/min. Chemical additions were performed at 24 hr and continued until 72 hr except for the colchicine and vincristine sulfate cultures in which the treatment was discontinued at 43 hr by the addition of fresh culture medium following centrifugation and washing of the cells. PBS was used as a carrier for each chemical except for diethylstilbestrol in which dimethyl sulfoxide (DMSO) was employed (final concentration 2.5 μl/ml). Cytochalasin B (final concentration 3 μg/ml) was prepared as described previously [Fenech and Morley, 1985] and was added at 44 hr. At 72 hr. the cells were centrifuged directly onto slides (48 g, 5 min) using a Leif bucket technique with one or more layers of filter paper (Whatman #3; Maidstone. England) to absorb the excess culture medium. After drying briefly. the cells were fixed in 100% methanol for 15 min. Slides were either used imme-

diately for kinetochore labeling or stored desiccated under an N_2 atmosphere at −20°C until use.

Antibody Labeling Procedure

Slides were placed in PBS-0.1% Tween for 5 min after which the excess fluid was drained. The antikinetochore antibody solution was diluted with an equal volume of PBS-0.1% Tween, and 50 μl of this dilution was placed on each slide. The slides were coverslipped and placed in a humidified box at 37°C. After 1 hr, the coverslips were removed. the slides were rinsed twice in PBS-0.1% Tween for 2 min each, and the excess fluid was drained. A 50-μl aliquot of fluoresceinated rabbit anti-human IgG previously diluted 1:120 with PBS-0.5% Tween was placed on slides, incubated for 1 hr. rinsed. and drained as before. Two drops of DAPI (2.5 μg/ml), in an antifade solution for fluorescent microscopy [Johnson and Nogueira Araujo, 1981], were added onto the slide using a Pasteur pipet. The light-sensitive nature of DAPI required that counterstaining be performed under subdued or yellow fluorescent lighting. The DAPI solution and remaining fluid were mixed gently with the coverslip, and the excess fluid and air bubbles were removed by blotting. The labeled slides were used immediately or stored in the dark at 4°C. Using this procedure, the labeling remained adequate for scoring for several days.

Scoring Procedure

The fluorescein and DAPI fluorochromes excite maximally at 488 and 355 nm, and emit at 520 and 450 nm, respectively. Through the simultaneous use of phase contrast and DAPI excitation, it was possible to identify both the cell membrane and the nuclei while scoring. Scoring procedures involved visualizing cells under both phase contrast and DAPI excitation until a binucleated cell with a micronucleus was observed. The filter setting was then changed so that the fluorescein-labeled kinetochores could be observed to determine whether the micronucleus contained a kinetochore.

Slides from each treatment were randomized and coded prior to scoring. Replicate cultures were established for each experiment except arsenite in which only one culture was available. The number of micronuclei at each dose level was determined by scoring 1,000 binucleated cells (i.e., 1,000 mitotic events) except at the vincristine sulfate 0.065 μM and the sodium arsenite 9 μM doses in which 929 and 350 cells were scored, respectively. Scoring was performed using a scoring program on an Apple II computer developed at Lawrence Livermore National Laboratory (Livermore, CA). Published criteria for micronuclei determinations [Countryman and Heddle, 1976] were followed with the following modifications: 1) binucleated cells containing any number of micronuclei were scored: 2) fluorescence intensity per unit area of scorable micronuclei was either more or less intense that of the main nuclei; 3) only micro. nuclei that were distinctly separate from the main nuclei and located within binucleate cells with intact cytoplasmic and nuclear membranes were scored. In addition, micronucleated cells were considered scorable for kinetochores only if the kinetochores in the main nuclei were distinctly visible.

A measurement of nuclear division for each treatment was determined by scoring the number of nuclei in 400 cells and calculating the nuclear division index:

$$NDI = \{[M1 + (2 \times M2) + (3 \times M3) + (4 \times M4)]/N\}$$

where M1–M4 represent the number of cells with one to four nuclei, respectively, and N is the total number of cells scored.

Statistical Analyses

Statistical analyses were performed using the Fisher exact test to compare the micronucleated cell and the kinetochore-positive micronucleated cell frequencies between control and treatment slides. Critical values were determined using a .05 probability of type I error.

RESULTS

The use of an antikinetochore antibody to determine the location of kinetochores within the nuclei and micronuclei of human lymphocytes is demonstrated in Figures 1 and 2. Figure 1a shows a binucleated lymphocyte with two micronuclei using the phase/DAPI filter setting. The same cell is shown in Figure 1b under fluorescein excitation and emission. Numerous kinetochores in the main nuclei and two kinetochores in each of the micronuclei are visible in this plane of focus. Figure 2 shows a binucleated cell with multiple micronuclei in which none of the induced micronuclei contain kinetochores. The number of kinetochores visible within a cell varies depending on the stage of the cell cycle, the microscope plane of focus, the overlap of individual spots, and the efficiency of antibody penetration. Rigorous attempts to identify and enumerate individual kinetochores resulted in the identification of 40–46 fluorescein spots per interphase nucleus in normal binucleated cells and 46 fluorescein spots in virtually all metaphase spreads from colcemid-arrested lymphocytes. Interestingly, in a high percentage of the smaller mononuclear cells (i.e., those not responding to PHA stimulation), only 12–15 bright fluorescein spots were observable (Fig. 2b). This may indicate a different centromeric organization in nondividing G_0 cells or a difference in chromatin structure interfering with epitope accessibility.

Three spindle-disrupting chemical agents were tested for their ability to induce cells with kinetochore-positive micronuclei. Colchicine and vincristine sulfate treatments induced the formation of micronucleated cells in a dose-re-

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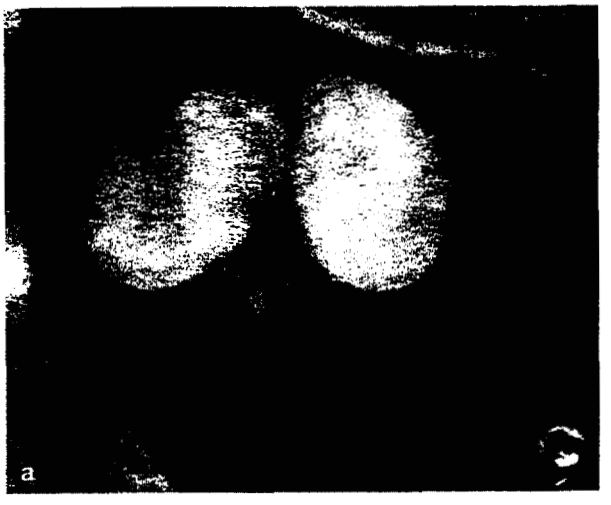

Fig. 1. A cytokinesis-blocked binucleated lymphocyte with two micronuclei photographed using (a) simultaneous phase contrast and DAPI excitation and emission wavelengths and (b) fluorescein excitation and emission wavelengths. Multiple kinetochores are visible in this plane of focus in each of the main nuclei and in the micronuclei (arrows).

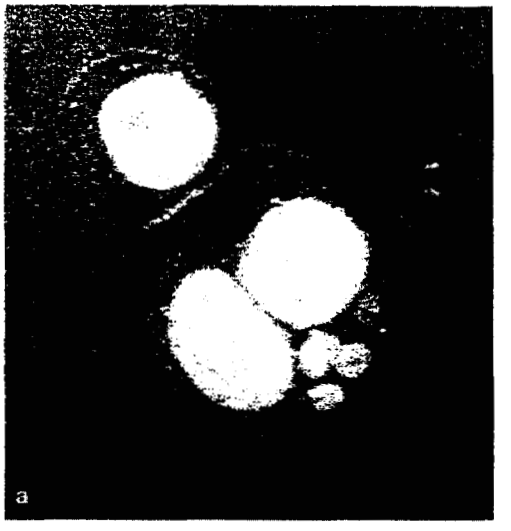

Fig. 2. A cytokinesis-blocked binucleated lymphocyte with four micronuclei and a mononucleated lymphocyte photographed using (a) simultaneous phase contrast and DAPI excitation and emission wavelengths and (b) fluorescein excitation and emission wavelengths. Fluoresceinated kinetochores are observed in the main nuclei of both the bi- and mononucleated cells but not in the micronuclei.

ted manner as illustrated in Figures 3 and 4, respectively,

Table I. A statistically significant increase in total micronucleated cells and kinetochore-positive micronucleated cells was seen at doses of 0.050 μM and greater for colchicine, and 0.026 μM and greater for vincristine sulfate. Although the lower doses of diethylstilbestrol exhibited minor increases above control values, an unambiguous statistically significant increase both in micronucleated cells and kinetochore-positive micronucleated cells was seen only at the 30-μM concentration of diethylstilbestrol (Fig. 5. Table I). The lines representing total micronucleated cells and the scorable kinetochore-positive cells for these three

TABLE 1. Distribution of Micronuclei Per Binucleated Cell

Agent/dose	No. of cells scored	Total no. of micronuclei	No. of micronuclei per binucleated cell						Probability[a]
			0	1	2	3	4	5+	
Colchicine									
0[b]	1,041	11	1,031	9	1	0	0	0	—
0.025	1,019	27	1,002	15	1	0	0	1	0.11
0.05	1,101	45	1,071	22	6	0	1	1	<.002
0.075	1,000	95	943	37	11	5	1	3	<.0001
0.1	1,012	195	914	49	33	19	5	3	<.0001
Vincristine sulfate									
0[b]	1,129	15	1,117	10	1	1	0	0	—
0.026	1,070	35	1,041	25	3	0	1	0	<.004
0.039	1,194	101	1,126	49	11	3	4	1	<.0001
0.052	1,014	73	954	51	7	1	0	1	<.0001
0.065	929	193	810	84	19	7	5	4	<.0001
Diethylstilbestrol									
0[b]	1,049	8	1,043	4	2	0	0	0	—
5	1,006	24	991	12	2	0	0	1	.03
10	1,001	15	990	8	2	1	0	0	.14
20	1,173	22	1,156	12	5	0	0	0	.03
30	1,003	57	957	38	5	3	0	0	<.0001
Sodium arsenite									
0[b]	1,000	8	993	6	1	0	0	0	—
3	1,000	33	974	13	0	2	1	0	<.001
6	1,000	87	926	65	6	2	1	0	<.0001
9	350	31	322	25	3	0	0	0	<.0001
Radiation									
0[c]	1,026	10	1,017	8	1	0	0	0	—
100	1,000	90	915	80	5	0	0	0	<.0001
200	1,000	286	757	209	26	7	1	0	<.0001
300	1,000	543	580	318	82	19	1	0	<.0001
400	1,000	917	409	353	182	56	8	2	<.0001

[a] Probability values are based upon a one-tailed Fisher exact test comparing the number of micronucleated cells per treatment with the respective control.
[b] Micromolar.
[c] Rads.

agents are nearly parallel, indicating that their effects are primarily aneupioidogenic and are observed at both high and low doses. In addition, the majority (77–93%) of the total micronuclei induced by these chemicals were kinetochore positive.

The clastogenic agents sodium arsenite and ionizing radiation resulted in a dose-related increase in micronucleated cells which was statistically significant at all doses when compared to controls (Figs. 6, 7, Table I). The data shown indicate that these agents are primarily clastogenic in nature as expected. However, at the higher doses for both sodium arsenite and ionizing radiation, small but significant increases in kinetochore-positive micronucleated cells were observed.

The distribution of micronuclei per binucleated cell for each agent and dose is shown in Table I. Twenty-two of the 24 distributions differed significantly from the Poisson, due to an excess of cells with multiple micronuclei. This excess indicates that the number of micronuclei per cell is not random; i.e., a cell with one micronucleus is more likely to contain additional micronuclei.

Table II shows the nuclear division data for each of these five agents. For each agent, a dose-related decrease in the nuclear division index was observed which was particularly apparent for cells that had received continuous chemical treatment.

An indication of the specificity of this assay can be estimated by the percentage of kinetochore-positive micronucleated cells relative to the total number of scorable micronucleated cells. These frequencies for colchicine, vincristine sulfate. and diethylstilbestrol treatments are 92, 87, and 76%, respectively. However, only 3 and 19% of the scorable micronucleated cells induced by ionizing radiation and sodium arsenite. respectively, contained kinetochore-positive micronuclei.

Occasionally. a micronucleated cell would be found in

TABLE II. Percent of Cells With Different Numbers of Nuclei

Treatment	Dose	Percent of cells with indicated number of nuclei				Nuclear division index[a]
		1	2	3	4	
Colchicine	0[b]	72	27	1	0	1.29
	0.025	59	37	3	1	1.46
	0.050	78	19	1	2	1.27
	0.075	85	15	0	0	1.16
	0.100	88	11	1	0	1.13
Vincristine sulfate	0[b]	66	28	3	3	1.43
	0.026	70	14	4	2	1.37
	0.039	81	17	2	0	1.21
	0.052	80	16	3	1	1.25
	0.065	87	12	1	0	1.15
Diethylstilbestrol	0[b]	54	38	4	4	1.59
	5	68	29	1	2	1.37
	10	75	13	1	1	1.30
	20	80	20	0	0	1.20
	30	94	6	0	0	1.07
	40	99	1	0	0	1.03
Sodium arsenite	0[b]	57	39	3	1	1.48
	3	82	17	1	0	1.18
	6	89	11	0	0	1.11
	9	98	2	0	0	1.02
	12	99	0	1	0	1.01
Radiation	0[c]	57	36	6	1	1.52
	100	59	37	3	1	1.47
	200	66	30	4	0	1.39
	300	65	32	2	1	1.40
	400	75	21	3	1	1.28

[a]Nuclear division index = [M1 + (2 × M2) + (3 × M3) + (4 × M4)]/N where M1–M4 are the number of cells with 1–4 nuclei, and N is the total number of cells scored.
[b]Micromolar.
[c]Rads.

which the kinetochore labeling was inadequate for scoring. The frequency of scorable micronucleated cells in the lymphocyte cultures varied by experiment, ranging from 85% for sodium arsenite-treated cells to 98% for radiation-treated cells.

Of 4,196 binucleated cells scored in control cultures (pooled from all experiments except diethylstilbestrol in which DMSO was used as a carrier), 38 were micronucleated (0.9%). Of these, 12 contained at least one kinetochore-positive micronucleus for a 0.0029 frequency of potentially aneuploid cells. Ninety-two percent (35/38) of the control micronucleated cells were considered scorable on the basis of kinetochore staining. These limited results from a single donor indicate that 34% (12/35) of the scorable micronucleated cells in the control cultures contained a kinetochore.

DISCUSSION

The use of a modified micronucleus assay using an anti-kinetochore antibody appears to have considerable promise as an assay to identify aneuploidogenic agents. The principal advantages are that it is a relatively rapid and simple assay that can utilize human cells, and can also be applied to established cell lines. Since it does not utilize metaphase spreads, but rather relies upon cells with intact cytoplasmic membranes, technical artifacts due to the loss of chromosomes that are common with standard cytogenetic techniques are not a problem. Furthermore, in contrast with other methods that determine whether a micronucleus contains an entire chromosome or a fragment [Heddle and Carrano, 1977; Yamamoto and Kikuchi. 1980; Valadaud-Barrieu, 1983; Banduhn and Obe, 1985; Hogstedt and Karlsson, 1985; Viaggi et al., 19871, the use of the kinetochore antibody allows the rapid determination of the composition of micronuclei without additional procedures (e.g., size measurements. autoradiography, DNA quantification).

The results presented in this paper indicate that the assay exhibits considerable specificity in distinguishing between aneuploidogenic and clastogenic agents. Each of the agents tested here resulted in dose-related increases in the formation of micronucleated cells with the possible exception of diethylstilbestrol in which a major increase in micronucleated cells was observed only at the highest dose. However,

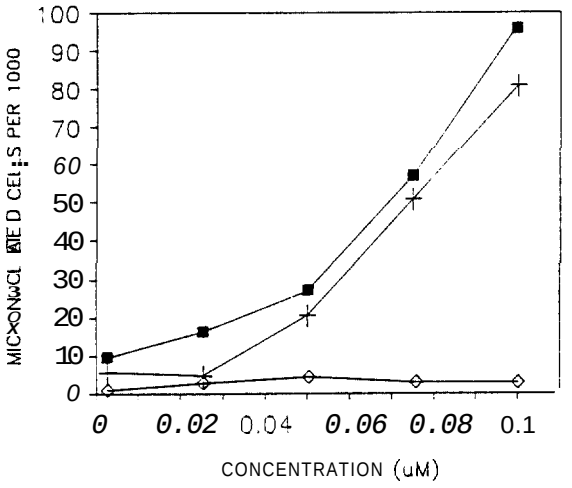

Fig. 3. Induction of micronucleated lymphocytes by various doses of colchicine: (■) total number of binucleated cells with micronuclei: (+) number of binucleated cells containing one or mom kinetochore-positive micronuclei: (O) number of binucleated cells containing no kinetochore-positive micronuclei. The difference between the total number of micronucleated cells and the sum of the kinetochore-positive and kinetochore-negative cells represents *the* number of unscorable micronucleated cells at each dose. The 0 dose has been slightly offset for clarity.

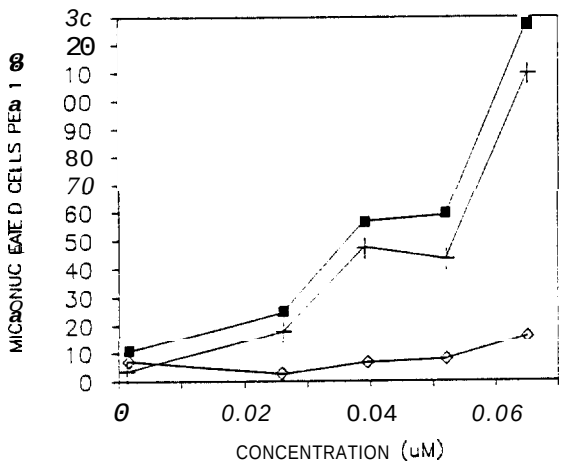

Fig. 4. Induction of micronucleated lymphocytes by various doses of vincristine sulfate. The key to the symbols is given in the legend to Figure 3.

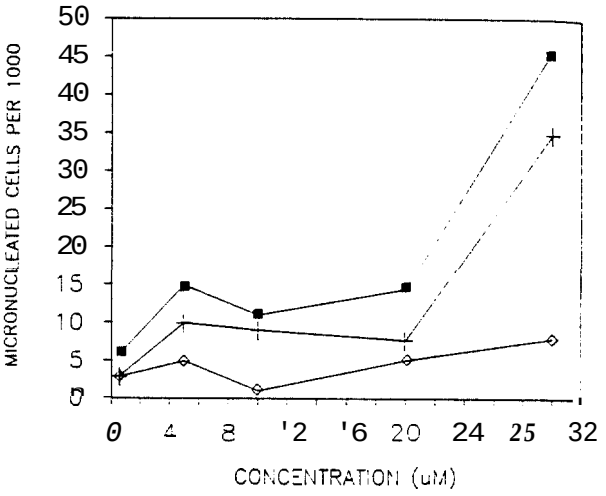

Fig. 5. Induction of micronucleated lymphocytes by various doses of diethylstilbestrol. The key to the symbols is given in the legend to Figure 3.

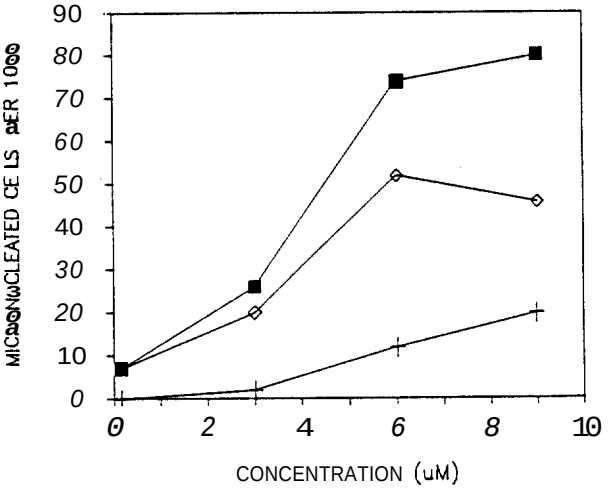

Fig. 6. Induction of micronucleated lymphocytes by various doses of sodium arsenite. The key to the symbols is given in the legend to Figure 3.

the frequencies of cells with one or more kinetochore-positive micronuclei induced by the aneuploidogens were substantially higher (76–92%) than the frequencies induced by the clastogens (3–19%). Based upon these results, the relative aneuploidogenic potential of a particular agent may be identifiable by the induced frequency of kinetochore-positive micronucleated cells.

Although the proportion of kinetochore-positive micronucleated cells was much higher for aneuploidogens than clastogens, both ionizing radiation and sodium arsenite

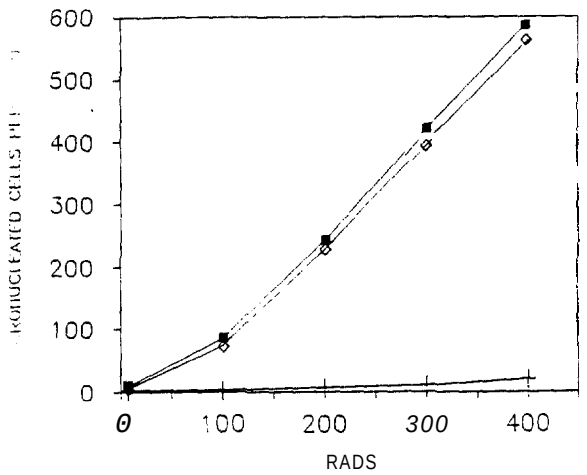

Fig. 7. Induction of micronucleated lymphocytes by various doses of ionizing radiation (^{137}Cs). The key to the symbols is given in the legend to Figure 3.

t. ..tments resulted in a significant increase in kinetochore-positive micronuclei at the higher dose levels. These results. suggest that these agents, although principally clastogenic [Heddle and Carrano, 1977; Wan et al., 19821, may exhibit some weak aneuploidogenic properties. In addition. the results observed with diethylstilbestrol suggest that weak clastogenicity may accompany diethylstilbestrol genotoxicity, an observation that agrees with previous observations [Howard et al., 1985]. The identification of a weak aneu- idogenic or clastogenic potential for these agents may be due to the relatively large sample sizes (1,000 cells per treatment) employed in these studies. This is considerably more than those used in standard cytogenetic analyses (typically, no more than **200** cells per treatment).

The major limitation of this assay is that only aneuploidogens that produce lagging chromosomes (and therefore micronuclei) can be identified. It is conceivable that certain classes of aneuploidogens such as those causing nondisjunction would not be detected by the antikinetochore approach. However, the results presented here and elsewhere indicate that micronuclei induction is a characteristic of many different classes of aneuploidogens [Heddle et al., 1983; Parry and Parry, 1987]. In addition, since the induced micronuclei have only a probability of segregating during cytokinesis with the daughter cell from which they were derived, the assay provides only an indirect measure of aneuploidy and aneuploidy induction. This principle is illustrated in Figure 8. Cells are scored at a cytokinesis-blocked stage, which is represented in the middle of the figure. If the cells were allowed to divide normally, the chromosome-containing micronucleus would segregate with only one of the daughter cells, resulting in several possible outcomes. If the micronucleus segregates with the daughter nucleus from which it was derived, the resulting daughter cells will be diploid, although one daughter cell will contain a micronucleus. Whether this type of cell will function normally or whether asynchronous DNA replication will occur as has been described previously [Obe and Beek, 1982] is unknown. If the micronucleus segregates with the daughter nucleus from which it did not originate, then both daughter cells will be aneuploid. The actual frequency of aneuploidy induction is unknown and will be largely dependent upon the nature and frequency of micronucleus segregation. In spite of these limitations, the modified micronucleus assay offers potential for identifying a wide variety of aneuploidogens.

One characteristic of the chemicals tested in this assay was that the observed dose-related increases in micronuclei frequencies were accompanied by decreases in cell growth and nuclear division. This effect can be readily seen in Tables I and II. For each chemical and, to a lesser extent, radiation, a considerable reduction in the nuclear division index and the percentage of binucleated cells was observed as micronuclei were induced by these agents. The extreme case of this phenomenon was observed in preliminary experiments with colchicine and vincristine sulfate in which the cells received a continuous treatment with these agents. Numerous cells were observed which apparently had been unable to divide resulting in the formation of mononuclear cells with multiple micronuclei. For these two chemicals, the use of 19-hr pulse treatments (24–43 hr following culture setup) enabled the partial release of cells from this block and allowed the formation of binucleate cells with micronuclei.

The use of the antikinetochore antibody to determine aneuploidy induction has a potential utility for a variety of disciplines including genetic toxicology, carcinogenesis, and teratology. Preliminary results from our laboratory have shown that this approach can be readily adapted for use in vitro with Chinese hamster ovary cells and mouse lymphocytes or in vivo with mouse erythrocytes. The combination of this antikinetochore approach with biomonitoring studies using cytokinesis-blocked lymphocytes offers an opportunity to observe aneuploidy induction in the cells of workers or patients exposed to genotoxic or chemotherapeutic agents.

These initial studies have shown that the use of an antikinetochore antibody combined with the chemical- and radiation-induced formation of micronuclei in cytokinesis-blocked human lymphocytes is capable of distinguishing between the aneuploidy-inducing and clastogenic potentials of a variety of agents. As such, this assay represents a novel approach to identify the aneuploidy-inducing potential of chemicals and other agents. Additional studies employing a wide variety of aneuploidogenic and clasrogenic agenrs ***will*** be required to properly validate this method as an assay for aneuploidy.

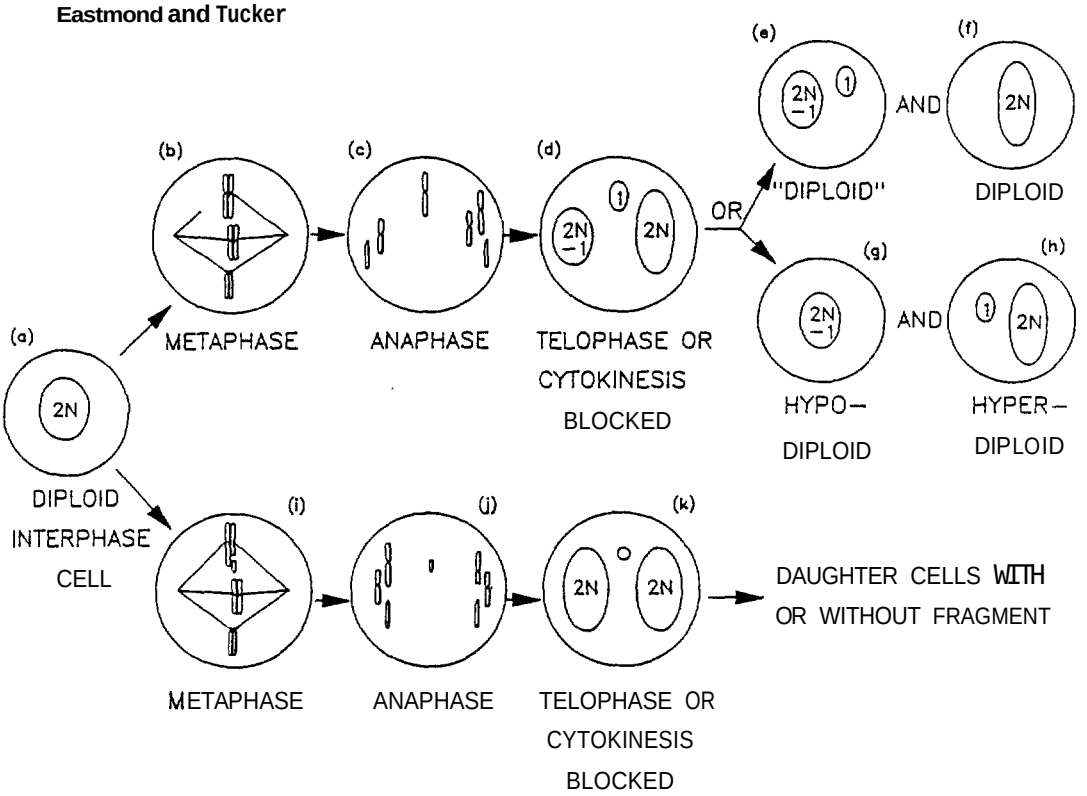

Fig. 8. Possible series of mitotic events resulting in micronucleated and aneuploid cells. Treatment of a normal diploid cell (a) with an aneuploidogen causes an interference with the mitotic spindle apparatus (b), which in turn results in a lagging chromosome during anaphase (c). During late telophase (d), this lagging chromosome forms a micronucleus. which may segregate with one of the daughter nuclei. The segregation of the micronucleus with the daughter nucleus from which it was derived results in two diploid daughter cells (e, f). The "diploid" cell (e) may not replicate normally (see text). The segregation of the micronucleus with the nucleus from which it did not originate results in two aneuploid daughter cells (g, h). Treatment of a normal diploid cell (a) with a clastogen results in a chromosome fragment (i), which lags during the anaphase (j) and results in a micronucleated cell during late telophase (k). The segregation of this micronucleus with either daughter nucleus results in daughter cells either containing or lacking the fragment. The presence of a kinetochore in the micronucleus of a cytokinesis-blocked cell (d) indicates a cell with a finite probability for aneuploidy assuming the cell had been allowed to divide normally.

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