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Genetics

Cytogenetic "rogue" cells: What is their frequency, origin, and evolutionary significance?

(clastogenic agents/spontaneous rearrangements/chromosomal damage)

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ABSTRACT **Among 102,170 cultured lymphocytes obtained from 9818 individuals from Hiroshima, Japan, aged 9 to 37 years and scored for chromosomal abnormalities, 24 cells that exhibited an extreme degree of damage were encountered. The damage consists of multiple dicentric and even tricentric chromosomes, as well as numerous fragments, many with the appearance of "double minutes." The occurrence of these cells was not correlated with parental exposure to the atomic bomb, age, sex, year, or season. They were nonrandomly distributed by individual. Such cells were originally described in South American Indians and have also been recorded in inhabitants of the United States and the United Kingdom; this appears to be a world-wide phenomenon. Their cause remains unknown, and it is not known whether they occur in other somatic and germ-line cells. Should the latter be the case and should the least damaged of these cells occasionally successfully complete mitosis and meiosis, the possible role of such cells in oncogenesis and evolution must be considered.**

In 1970 we reported (1) that, in studies of lymphocytes cultured from blood samples obtained from 49 apparently normal, quite unacculturated Yanomama Indians living in South America, we observed that about 1 in 200 of the cells exhibited an extreme collection of chromosomal abnormalities (dicentrics and tricentrics) plus scattered fragments. In two subsequent years, the frequency of such cells was much lower, about 1 in 5000 (2). In the original observation, the frequency of damaged cells per individual was not uniform, the observations departing grossly from a Poisson distribution. In a review in 1982, Cowell (3) pointed out that the "scattered fragments" we had encountered resembled the "double minutes" seen in the cells of some patients whose malignancies have been treated with radiation or chemical agents, notable among the latter being methotrexate. However, unlike the double minutes seen following cancer chemotherapy or the treatment of cultured cells with methotrexate, these double minutes would all seem to have arisen in a single cell generation. Because of the decrease in these cells over a two-year period, we favored the explanation that they were a transient manifestation of a tropical viral infection, but there was no supporting evidence for this suggestion.

The exotic nature of the population in which the finding was first encountered was scarcely conducive to thinking of this as a general phenomenon. Now, however, similar findings of very rare, complexly abnormal cells have been reported from three other laboratories. Hsu (4) pictures one such metaphase, encountered in a lymphocyte culture of a normal person whose spontaneous chromosome breakage frequency was otherwise low. Fox *et al.* (5) observed among specimens from 153 commercial and sports divers studied in the United Kingdom, from each of whom 100 cultured lymphocytes were examined, one or more such cells in the preparations from each of 6 men. No such cells were observed in 127 controls. Tawn *et al.* (6), in a study scoring 200 cultured lymphocytes from each of 12 presumably normal young subjects from the United Kingdom (10 men and 2 women), found such cells in 2 men; when the scoring of the preparations from these 2 men was extended to 500 cells, there were 4 such cells from 1 man and 5 from the other man. When the two persons were restudied 3 months later, among 500 cells scored from each there were no such cells. We suspect that others who have encountered these cells have not reported them because of their bizarre and inexplicable nature.

In this communication, we report on the occurrence of this phenomenon in still another population, the Japanese. The presence of such cells in normal Japanese individuals has already been briefly alluded to by Awa *et al.* (7), who observed among 24,414 cells cultured from adults with no known clastogenic experience, 5 cells "containing more than five exchange aberrations of unidentifiable nature" (≈1 per 5000 cells). Here we describe observations on the frequency of these cells in preparations from 9818 children of proximally and distally exposed survivors of the atomic bomb, examined in the course of studies of the cytogenetic effects of these weapons.

MATERIAL AND METHODS

The population studied is about evenly divided between the children of a group of "proximally exposed" survivors of the atomic bombing of Hiroshima (within 2000 m of the hypocenter) and the children of a group of distally exposed survivors (>2500 m from the hypocenter). The proximally exposed survivors received from 1 rem (1 rem = 0.01 S-v) of radiation up to the maximum consistent with survival; the distally exposed parents received essentially no radiation at the time of the bombing. These children were being studied in a search for evidence of transmitted chromosomal damage (8, 9); the findings to be described here are an incidental observation that, as we will show, is unrelated to the radiation history of the parents.

Venous blood samples were obtained in the usual fashion with 0.1 ml of 1000 international units of sodium heparin/ml added to 2–3 ml of blood as anticoagulant. For culture, 2 ml of whole blood was combined with 10 ml of MEM (modified Eagle's medium) plus 0.3 g of glutamine/liter and 2 ml of heat-inactivated fetal bovine serum. Just prior to incubation, 0.1 ml of phytohemagglutinin (10 mg/ml, Wellcome) was added to the preparation. At 50 hr of incubation, 0.1 ml of 0.4 μg of colchicine/ml was added to the preparation, and incubation was continued for another 2 hr. Cells were harvested and treated with a hypotonic solution (a mixture of 1 part of 1% sodium citrate and 1 part of 0.075 M KCl, then fixed with a methanol/acetic acid mixture (3:1, vol/vol); the preparation was flame dried and stained with standard

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Giesma solution [2% (wt/vol), pH 7.4–7.5]. Usually 10 well-spread metaphases were scored from each subject [see Awa *et al.* (9) for further discussion of methods].

RESULTS

Over an 18-year period a total of 102,170 cells derived from 9818 persons have been examined (Table 1). Twenty-four of these cells exhibited the extreme degree of chromosomal damage pictured in Fig. 1. We have termed these rogue cells. The findings characteristic of the remaining cells in these preparations have been, in part, described by Awa *et al.* (9); there are no intergradations between these highly abnormal cells and cells exhibiting what might be termed the usual chromosomal damage (as well as numerical aberrations) encountered in persons not exposed to a chromoclastic agent. Although it is difficult to be precise, there appear to be about 46 centromeric regions in each of these abnormal cells, i.e., they are essentially diploid. The number of (paired) fragments is difficult to score accurately, but we estimate that it ranges from 2 to >10 per cell. The damage exhibited by the average rogue cell in the present series is at least as great as that encountered in Amerindians by Bloom *et al.* (1) or as pictured by Fox *et al.* (5).

In the original series, among the 24 persons exhibiting these cells, only 1 such abnormal cell was observed among the 10 cells scored per individual (Table 2). (Early in the series, sometimes more than 10 cells from one individual were "routinely" scored.) Following the observation of a rogue cell, additional cells were scored, where possible, in those same persons up to the total number indicated in Table 2. In six persons additional rogue cells were observed. Thus, among the additional cells scored because of the observation of a single rogue cell among the 10 routinely scored, there were 7 rogue cells among 2138 cells. This frequency is clearly higher than the originally observed frequency ($\chi^2 = 65.1$, df $= 1$, $P < 0.001$) and indicates a nonrandom distribution of the phenomenon among individuals. This confirms the experience of Bloom *et al.* (1) and Tawn *et al.* (6).

EPIDEMIOLOGICAL ANALYSES

The data can be considered from a number of epidemiological standpoints, as follows: (*i*) Sex. The series from which individuals exhibiting these cells is drawn is approximately evenly divided as to sex (4732 males and 5086 females); there is a borderline preponderance of males among those exhibiting this finding (16 males and 8 females, $\chi^2 = 3.30$, df $= 1$, $0.05 < P < 0.10$). (*ii*) Age. The mean age of persons exhibiting rogue cells is 24.5 ± 6.2 years (mean ± SD); the mean age of persons without such cells is 23.4 ± 6.3 years. This observation, in conjunction with the earlier observation (7), would seem to exclude an age effect. (*iii*) Exposure of parents to atomic bombs. For the total sample, one or both parents of the child had been proximally exposed to the atomic bomb in 4700 cases, and distally exposed in 5094 cases. The corresponding figures for the children exhibiting these cells are 11 and 13 rogue cells ($\chi^2 = 0.06$, df $= 1$, $0.80 < P < 0.90$). (*iv*) Secular trend. We have searched for a secular trend in two ways. Table 3 presents the findings with reference to year of study. The data of Table 3 have been grouped by year of study in such a way as to yield four samples of approximately equal size. The result is shown in Table 4. There is no evidence of heterogeneity by year. A second approach is to examine the interval in the series between individuals exhibiting positive findings. This can be extracted from data in Table 2 because the samples were numbered consecutively as acquired. The results are plotted in Fig. 2. There is no evidence for a grouping; the data conform to expectation based on a Poisson process ($\chi^2 = 2.65$, df $= 2$, $0.20 < P < 0.30$). (*v*) Season. The possibility of a seasonal effect has been examined by grouping the positive findings by month of sampling as follows: December–February, March–May, June–August, and September–November. The results are 5, 10, 4, and 5 rogue cells, respectively. This does not differ from the distribution for the total sample, for which the corresponding figures are 2449, 2773, 2232, and 2364 ($\chi^2 = 2.20$, df $= 3$, $0.50 < P < 0.70$). (*vi*) Storage effect. The blood samples were usually processed immediately upon collection, except those collected at the "Thursday night clinic" (the only night clinic). After they had been mixed with the culture medium, these Thursday night samples were refrigerated for 36–40 hr before processing. Specimens not collected at the Thursday night clinic were usually processed the day of collection. Analyzing the data, we find that 22 of the 24 samples exhibiting rogue cells (92%) were collected on a Thursday night. On the other hand, analysis of all the samples for time of collection reveals that 4390 of 9818 (45%) were collected on Thursdays ($\chi^2 = 21.5$, df $= 1$, $P < 0.001$). This "epidemiological clue" is difficult to evaluate. A small scale experiment, involving refrigerating blood samples for an additional 24 hr, yielded no increase in minor chromosomal aberrations over the laboratory standard in 4179 scored cells nor were any rogue cells observed. While a storage effect may be implicated, it can scarcely be regarded as causative *per se*, but at most as triggering this phenomenon in sensitive cells.

DISCUSSION

It now seems clear that the rogue cell phenomenon is widespread, having been recorded in North and South America, England, and Japan. It is possible we have missed other, passing references to such cells in the voluminous literature of human cytogenetics. The data strongly suggest that the phenomenon is nonrandomly distributed among individuals, in whom it peaks and then declines.

The cause of these extraordinary cells remains completely mysterious. Somewhat comparable cells have been produced experimentally by temporary extreme folic acid and/or thymine deficiency (10–12), but the aberrant karyotypes result from multiple chromatid rather than chromosome breaks. It seems beyond consideration that the cells we are describing could be primarily artifacts of the culture technique. None of the epidemiological clues available to us—effect of sex, age at examination, or year or season of examination—are helpful. The biological significance of the

Table 1. Frequency of occurrence of rogue cells in 102,170 arrested metaphase preparations observed from Hiroshima Japanese aged 9 to 37

Metaphase preparations			Rogue cells			
Sex	No.	Cells examined, no.	No.	Rate, no. per cell	Carriers, no.	Rate, no. per person
Males	4732	49,420	16	0.33×10^{-3}	16	3.38×10^{-3}
Females	5086	52,750	8	0.15×10^{-3}	8	1.57×10^{-3}
Total	9818	102,170	24	0.23×10^{-3}	24	2.44×10^{-3}

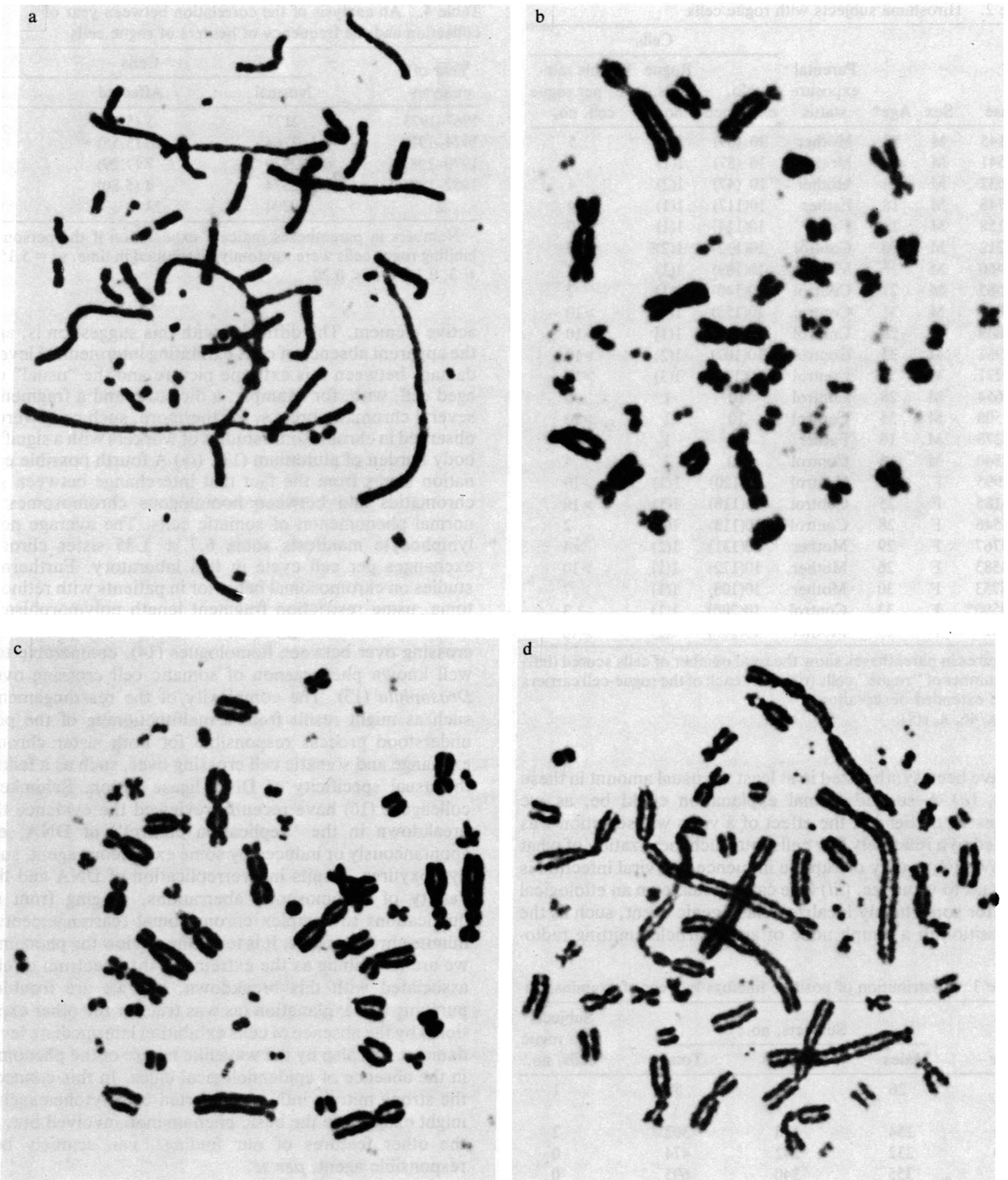

FIG. 1. Photomicrographs of arrested metaphases demonstrating rogue cells. Source of specimens as follows: (*a*) FH 3158 (male, aged 19), (*b*) FH 6231 (male, aged 31), (*c*) and (*d*) FH 9824 (female, aged 13).

"Thursday night clinic" effect mentioned above is obscure. Although specific culture conditions might to some extent trigger or intensify the phenomenon, given the experience and standardization of cytogenetic procedures in the various laboratories in which the phenomenon has been encountered, it is difficult to attribute the nonrandomness of the finding solely to variations in the way individual samples are processed. The cells that have been described and pictured thus far could very seldom complete a cell division without severe aneuploidy in the daughter cells. Accordingly, it seems almost certain the chromosomal events leading to the findings occurred in the interval following the last successful cell division. The striking difference between these cells and the other cells of the same person suggests the action of some highly localized factor. Among the possible explanations are the following: (*i*) Hsu (4) suggested "a defective DNA synthesis system, probably as the result of a mutation." We have difficulty visualizing a single mutation with such an instantaneously deleterious effect, even if it occurs in a cell that is already heterozygous for this mutation, so that the defective cell is homozygous deficient. Furthermore, the response to phytohemagglutinin is normal, and DNA appears

Table 2. Hiroshima subjects with rogue cells

Case	Sex	Age*	Parental exposure status	Cells: No. examined	Cells: Rogue cells, no.	Cells: Double minutes per rogue cell, no.
FH0145	M	18	Mother	30 (69)	1(1)	5
FH0541	M	18	Mother	10 (37)	1(1)	4
FH0632	M	20	Mother	10 (47)	1(2)	4
FH2748	M	18	Father	10(117)	1(1)	4
FH3158	M	19	Father	10(114)	1(1)	>10
FH3212	M	20	Control	10(132)	1(2)	>10
FH3460	M	26	Mother	10(109)	1(1)	>7
FH3585	M	27	Control	10(140)	1(1)	2
FH5030	M	31	Control	10(153)	1(1)	>10
FH5839	M	24	Control	10(105)	1(1)	>10
FH5951	M	33	Control	10(107)	1(2)	>10
FH6231	M	31	Control	10(126)	1(3)	>10
FH6654	M	28	Control	10	1	6
FH7500	M	35	Control	10	1	>10
FH8278	M	16	Father	10	1	3
FH8540	M	15	Control	10	1	4
FH2995	F	23	Control	10(120)	1(1)	>10
FH3185	F	25	Control	10(118)	1(1)	>10
FH3546	F	28	Control	10(113)	1(1)	2
FH3767	F	29	Mother	10(131)	1(2)	>4
FH4583	F	26	Mother	10(112)	1(1)	>10
FH5753	F	30	Mother	10(108)	1(1)	7
FH8590*	F	33	Control	10(200)	1(1)	3
FH9824	F	15	Mother	10(200)	1(2)	>10

Figures in parentheses show the total number of cells scored (left) and number of "rogue" cells (right) for each of the rogue-cell carriers in the extended observation.
*45, x/46, x, r(x).

to have been synthesized in at least the usual amount in these cells. (*ii*) A second formal explanation could be, as we suggested earlier (1), the effect of a virus whose action was limited to a relatively few cells, but such localization of what is obviously a very disruptive influence in a viral infection is difficult to visualize. (*iii*) One can speculate on an etiological role for some highly localized clastogenic agent, such as the deposition in a lymph node of an α-particle-emitting radio-

Table 3. Distribution of positive findings by year of examination

Year	Subjects, no.: Males	Subjects, no.: Females	Subjects, no.: Total	Subjects with rogue cells, no.
1967	26	32	58	1
1968				
1969	154	148	302	2
1970	232	242	474	0
1971	355	340	695	0
1972	118	109	227	0
1973	193	231	424	0
1974	115	149	264	0
1975	98	129	227	1
1976	253	294	547	4
1977	290	275	565	4
1978	299	372	671	1
1979	462	500	962	1
1980	423	548	971	4
1981	499	554	1053	2
1982	577	582	1159	3
1983	489	416	905	0
1984	149	165	314	1
Total	4732	5086	9818	24

Table 4. An analysis of the correlation between year of collection and the frequency of bearers of rogue cells

Year of analysis	Cells: Normal	Cells: Affected	Total
1967–1973	2177	3 (5.32)	2180
1974–1978	2264	10 (5.55)	2274
1979–1981	2979	7 (7.29)	2986
1982–1984	2374	4 (5.80)	2378
Σ	9794	24	9818

Numbers in parentheses indicate expectation if the persons exhibiting rogue cells were randomly distributed in time. $\chi^2 = 5.156$. df = 3. $0.10 < P < 0.20$.

active element. The difficulty with this suggestion is, again, the apparent absence of cells exhibiting intermediate levels of damage between this extreme picture and the "usual" damaged cell, with, for example, a dicentric and a fragment, or several chromatid breaks. Furthermore, such cells were not observed in chromosome studies of workers with a significant body burden of plutonium (13). (*iv*) A fourth possible explanation stems from the fact that interchange between sister chromatids and between homologous chromosomes is a normal phenomenon of somatic cells. The average normal lymphocyte manifests some 6.7 ± 1.35 sister chromatid exchanges per cell cycle in this laboratory. Furthermore, studies on chromosomal behavior in patients with retinoblastoma, using restriction fragment length polymorphisms on chromosome 13, suggest the occurrence of somatic cell crossing over between homologues (14), comparable to the well known phenomenon of somatic cell crossing over in *Drosophila* (15). The complexity of the rearrangements is such as might result from a malfunctioning of the poorly understood process responsible for both sister chromatid exchange and somatic cell crossing over, such as a failure in the usual specificity of DNA ligase action. Schimke and colleagues (16) have recently reviewed the evidence that a breakdown in the "replication control" of DNA, either spontaneously or induced by some extraneous agent, such as hydroxyurea, results in overreplication of DNA and thus a variety of chromosomal aberrations, ranging from small duplications to complex chromosomal rearrangements and minute chromosomes. It is tempting to view the phenomenon we are describing as the extreme in the spectrum of effects associated with this breakdown, but we are troubled in pursuing this explanation (as was true for the other explanations) by the absence of cells exhibiting intermediate levels of damage, and also by the wavelike nature of the phenomenon in the absence of epidemiological clues. In this connection, the strong mitotic influence exerted by phytohemagglutinin might exaggerate the basic phenomenon involved but, given the other features of our findings, can scarcely be the responsible agent, *per se*.

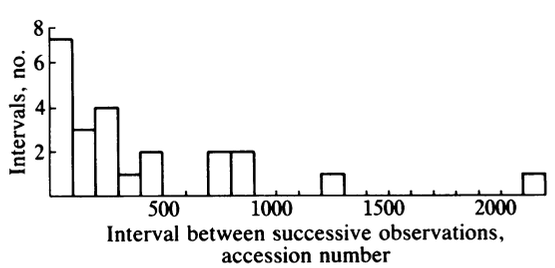

FIG. 2. Interval between encountering individuals exhibiting one or more rogue cells, as measured by consecutively assigned sample accession numbers.

The frequency of this phenomenon in the lymphoid lineage at the time of a "wave" cannot be estimated with any accuracy at present. There must be a rather low probability that even the least striking of these cells can successfully complete the mitotic process, certainly less than one percent. Thus, if these cells are responding to the normal mitotic stimulus (and not simply accumulating), then in persons in whom the rogue cell phenomenon is peaking the frequency of origin of such cells should be substantially higher than the one in several hundred observed by various investigators.

Whether this phenomenon occurs in other types of somatic cells and what its long-range consequences are can only at present be the subject of speculation. Malignant cells of different types often manifest complex and somewhat specific patterns of chromosomal rearrangement. The possibility must be considered that the small fraction of these rogue cells that survive their first mitotic division may become, in some instances by virtue of rearrangement-activated oncogenes, the basis for a malignant clone of cells.

It is not yet established whether the phenomenon occurs in germ cells, although the rare reports of children with multiple chromosomal abnormalities (for summary see ref. 17) may suggest this to be the case. If it is a phenomenon of the germ line, one could visualize in the population of damaged cells a spectrum of severity, with some of the least damaged cells able to navigate meiosis successfully. If the resulting gamete possessed an unbalanced chromosomal composition, the result would be a grossly defective child; some of these might survive to term. In the rare case of gametes that emerge from this event with a balanced genome, the result could be the type of chromosomal reorganization that figures so largely in evolutionary speculation. Given that the phenomenon occurs in bursts, it is difficult to estimate an average frequency of germ cells resulting from this event. But were the event of the same order of frequency in the spermatogonia as may be the case for somatic cells in this series and should even 10^{-2} of these cells (the less damaged) successfully complete meiosis and emerge with a balanced genome, this would constitute a frequency to be reckoned with in evolutionary thought.

Schimke and colleagues (16) have expressed similar thoughts concerning a role of a breakdown in the replication control of DNA in carcinogenesis and evolution. It would be of extreme interest to establish whether the phenomenon we are describing is in fact the extreme in the spectrum of effects to be associated with this breakdown.

How general the rogue cell phenomenon is throughout the animal and plant kingdom remains to be determined. No other species has been subjected to the amount of karyotyping of presumably normal cells as the human species. A phenomenon of this rarity could easily have escaped attention in even such genetically well studied organisms as *Drosophila* and the mouse. Burdensome though the undertaking would be, it would be of great interest to generate comparable data from the mouse. Although the nature of the mouse karyotype has posed difficulties for classical cytogenetics, the phenomenon under discussion should be readily recognizable.

The appearance of numerous "double minutes" in cells, *in vivo* or *in vitro*, that have not been subjected to a clastogen is commonly interpreted as the result of cumulative amplification of specific chromosomal segments in response to some noxious agent. In this instance, these double minutes have come into existence in a single generation, in a cell whose exposure to a noxious agent can scarcely be as different from all the rest of the cells as this interpretation would imply. A considerable fraction must be the type of fragment that results at the time of formation of a dicentric. Whether in addition some are the consequence of an abortive replication procedure remains to be determined. Studies of DNA content should be helpful in deciding this question.

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