

Chromosome Aberrations among the Yanomama Indians*

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Abstract. The chromosomes of leucocytes cultured from the peripheral blood of 49 primitive Yanomama Indians of Venezuela were studied to determine the types and frequencies of aberrations in a human population not exposed to the same exogenous agents as civilized man. In all but one instance, 100 cells per individual were scored. In 13 cases, we found one or more cells with multiple complex breaks and rearrangements, represented by tetracentric, tricentric, and numerous dicentric chromosomes. From the standpoint of chromosomal damage, these cells are among the most abnormal cells yet described *in vivo* in man, and were not seen in the controls. There was also a higher than expected frequency of cells with an isolated structural aberration in both Indians and controls. This may be the result of a 24- to 48-hour delay in the initiation of culture. The cause of the more extensive damage to some cells remains to be determined.

Leucocytes from presumably normal subjects occasionally show chromosomal aberrations, the types and frequencies of which are age-dependent.^{1,2} The causes of this damage are unknown, but cumulative exposure to a variety of agents in the environment of man may be an important factor. More specifically, some fraction of this damage might be due to the exposure of civilized man to a wide variety of chemical and physical mutagens. Accordingly, we thought it of interest to attempt to establish base line cytogenetic data from a truly primitive population, with no known exposure to medical radiation, food preservatives, pesticides, etc. This paper is a preliminary report of results of studies on 49 Yanomama Indians, and a group of controls.

Materials and Methods. Subjects: The Yanomama are a tribe of approximately 10,000 persons distributed among some 100 villages located in Southern Venezuela and Northern Brazil, in the Upper Orinoco River drainage basin, and in the drainage basin of some of the principal northern tributaries of the Amazon River. Most of the Yanomama are to be found between latitudes 1° to 5° north and longitudes 62° to 65°30" west. There is a subsistence economy, based on hunting, gathering, and slash-and-burn agriculture, in which the cooking banana (*Musa paradisiaca*) figures prominently. Their material culture is quite simple.^{3,4} Although their tribal area has been traversed by a number of expeditions, sustained contacts with non-Indians (missionaries) date back less than 20 years, and even today there are many villages not yet reached by non-Indians. This report will be limited largely to the findings in subjects from two villages located near the Venezuelan-Brazilian border (approx. 2°50" N, 64°20" W, and 3°0" N, 64°20" W). Access to the area was by air strips located in patches of

natural savannah. Both villages were visited in the course of multidisciplinary studies on these Indians.^{5,6}

Blood cultures: Venous blood samples for leucocyte culture were drawn into 10-ml heparinized vacutainers, immediately chilled in a portable refrigerator, and flown at 5–10°C to a temporary cytogenetics laboratory established at the Venezuelan Institute of Scientific Investigation, near Caracas. Members of the expedition provided control blood samples, drawn in the field and handled in the same way as the Indian bloods. All specimens reached the laboratory within 40 hr of the venipuncture, after which time the leucocytes were immediately separated and put into culture.

Cultures were established by the method of Bloom and Iida,⁷ using a 46- to 50-hr culture time. Slides were coded, and then brought back to Ann Arbor where they were analyzed. Single chromatid and isochromatid breaks were scored as such if there was displacement of the distal portion of the chromatid(s). When the chromosome of origin of an acentric fragment could not be determined, it was called a free fragment. Multicentric chromosomes were scored in accordance with the number of centromeres, while ring chromosomes were classified as either centric or acentric. Fragments accompanying multicentrics or rings were not scored separately. Translocations were generally detectable by the elongated long or short arm of the affected chromosome. Inversions showed displacement of the centromere. Other abnormalities noted were chromatid exchanges, breaks at the centromere, and deletions, which were usually terminal.

Of the 49 Indians successfully cultured, 32 were males, 17 were females. There were eight culture failures. Two of four control specimens obtained in the jungle from members of the expedition failed to grow. Accordingly, control data gathered in the study of the survivors of the atomic bombings of Hiroshima and Nagasaki were also used initially for comparison. Essentially the same culture techniques and approach to microscopy had been employed in obtaining the Japanese data as the present data, except for (a) the delay in the initiation of these cultures, and (b) the culture time itself.^{2,8} The cells of the A-bomb survivors were cultured for 72 hr. In a comparison of 2-day versus 3-day cultures, the frequency of observed aberrations was found to be slightly lower at the shorter culture time.⁹ We shall return to the question of the possible effect of the delay in culture initiation.

Results. One hundred cells were examined from 48 of the 49 subjects, with 75 cells being examined from the other subject. Each individual had a modal chromosome number of 46, and a normal karyotype. As is shown in Table 1, the variation in chromosome number was minimal, ranging from 96.7% of cells with 46 chromosomes among the Indian males to 97.1% among the Indian females. The "in-jungle" controls had 97.0% of cells at 46, while the Japanese control cells had 95.6%.

The numbers and percentages of cells with aberrations are given by type in Table 1. For virtually all types of aberrations, the Yanomama Indians exhibited an increase above the Japanese controls. For example, the controls had 0.65% of cells with a single chromatid break and 0.14% of cells with an isochromatid break. In the Yanomama Indians, on the other hand, 2.30% of the cells had single chromatid breaks and 0.68% had isochromatid breaks. If we include the free fragments with the isochromatid breaks, the controls had 0.20% per person, while the Indians had 1.07% per person. Isolated dicentrics or rings were seen in 5 of the 4875 Indian cells examined, or in 0.10% of cells. Among controls, only a single dicentric was seen in 16,035 cells, or in 0.006% of cells. No translocations or inversions were seen among the Indians, while those eight aberrations in the Japanese controls occurred in persons in the 7th and 8th decades of life. Eleven cells with chromatid exchanges or breaks at the

TABLE 1. *Cytogenetic findings in 49 Yanomama Indians from two villages in Venezuela.*

	Males	Females
Number of cases	32	17
Number of cells examined	3175	1700
Cells with 46 chromosomes (%)	96.7	97.1
Number (and percent) of cells with one or more aberrations		
Total	139 (4.38)	61 (3.59)
Single chromatid breaks	80 (2.52)	32 (1.88)
Isochromatid breaks	20 (0.63)	13 (0.76)
Free fragments	13 (0.41)	6 (0.35)
Dicentrics	3 (0.09)	1 (0.06)
Rings	1 (0.03)	0 (0.00)
Translocations, inversions	0 (0.00)	0 (0.00)
Chromatid exchanges	3 (0.09)	4 (0.24)
Centromere breaks	2 (0.06)	2 (0.12)
Number (and percent) of complex cells*	17 (0.54)	4 (0.24)

† Data obtained using same method of culture but with no delay in initiation of the 72-hr cultures.

* Complex cells include those with multiple, exchange-type aberrations (see Figs. 1 and 2).

centromere were seen among the Indians, a frequency of 0.22%; these were seen in 0.05% of control cells. Finally, there were cells which we have classified as complex. These cells differed from those described above in that they contained multiple exchange-type aberrations (see Figs. 1 and 2). These cells have not been previously described *in vivo* in presumptively normal humans, and were not seen in either the Japanese controls or the "in-jungle" investigators.

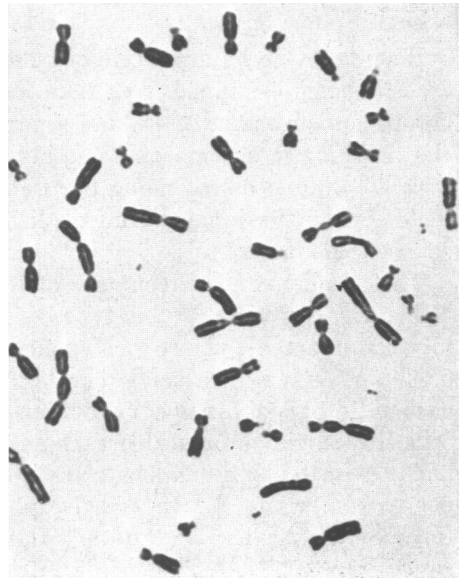
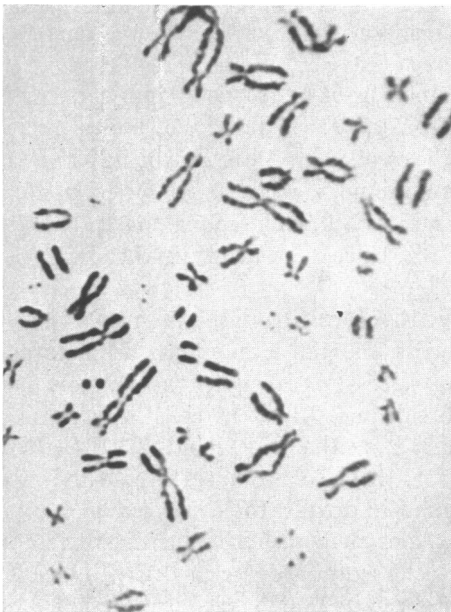


FIG. 1.—Complex metaphase from the cultured leucocytes of a 25-yr-old Yanomama male, with multiple dicentrics, a tricentric, and many fragments present.

FIG. 2.—Complex metaphase from the cultured leucocytes of a 45-yr-old Yanomama male, with multiple dicentrics and fragments.

TABLE 1. (continued)

Totals	Jungle controls	Japanese controls*
49	2	174
4875	250	16,035
96.8	97.0	95.6
Number (and per cent) of cells with one or more aberrations		
200 (4.10)	5 (2.00)	157 (1.0)
112 (2.30)	4 (1.60)	105 (0.65)
33 (0.68)	1 (0.40)	23 (0.14)
19 (0.39)	0 (0.00)	10 (0.06)
4 (0.08)	0 (0.00)	1 (0.006)
1 (0.02)	0 (0.00)	0 (0.0)
0 (0.00)	0 (0.00)	8 (0.05)
7 (0.14)	0 (0.00)	5 (0.03)
4 (0.08)	0 (0.00)	5 (0.03)
21 (0.43)	0 (0.00)	0 (0.0)

The two "in-jungle" controls exhibited a frequency of aberrations (2.0%) intermediate between the Indians and the Japanese controls. This figure is based on 150 cells from one control and on 100 cells from the other. Because of this paucity of controls, additional control bloods were drawn from the four Michigan members of the expedition upon the return of the expedition from the field. The leucocytes of these samples were separated and immediately cultured. One hundred cells were scored from each preparation, and the results are shown in Table 2. It should be noted that these four controls were still prophy-

TABLE 2. Control studies on four Caucasian males.

No. cells examined	Experiment	Experiment No. 2†			
	No. 1*	A	B	C	D
Cells with 46 chromosomes (%)	400	400	400	400	400
	96.0	96.8	94.3	97.0	90.8
	Number of cells with one or more aberrations				
Total	7 (1.75%)	5 (1.25%)	17 (4.25%)	13 (3.25%)	19 (4.75%)
Single chromatid breaks	1 (0.25)	3 (0.75)	5 (1.25)	8 (2.00)	8 (2.00)
Isochromatid breaks	4 (1.00)	1 (0.25)	4 (1.00)	4 (1.00)	1 (0.25)
Free fragments	1 (0.25)	0 (0.00)	2 (0.50)	0 (0.00)	5 (1.25)
Dicentric	0 (0.00)	0 (0.00)	5 (1.25)	0 (0.00)	3 (0.75)
Rings	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.25)
Translocations, inversions	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Chromatid exchanges	0 (0.00)	0 (0.00)	1 (0.25)	0 (0.00)	0 (0.00)
Centromere breaks	0 (0.00)	1 (0.25)	0 (0.00)	1 (0.25)	1 (0.25)
No. of complex cells	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

* Four bloods drawn while the subjects were on the antimalarial Camoprime. These cells were set into culture immediately.

† Columns A and B are data obtained after the four subjects had stopped Camoprime, with A cells cultured immediately, B cells cultured after a 48-hr delay. Columns C and D are data obtained after the four subjects had resumed Camoprime for 3 weeks. C cells were cultured without delay, D cells after 48-hr refrigeration at 8°C.

TABLE 3. *Distribution of cells with any kind of break, by age and sex of the Yanomama Indians.*

Age (yr.)	No. cells examined	Males	
		No. complex cells	No. cells with aberrations
0-10	700	4	23
11-20	675	2	14
21-30	700	2	35
31-40	200	4	11
41-50	400	2	11
51-60	500	3	35
61-70	0	0	0
Totals	3175	17	129

lactically on the antimalarial Camoprime¹⁰ at the time the specimens were obtained. The frequency of cells with any aberration (1.75%) was again intermediate between that of the Japanese and the Indians.

To test for the possible chromosomal effects of the antimalarial, but especially to determine any effect of the delay in initiation of cultures, the following experiment was then performed: Blood samples were drawn, again in Ann Arbor, from each of the four members of the expedition. Each sample was divided into two aliquots: the leucocytes from one aliquot were cultured immediately; the leucocytes from the other were cultured after being stored at 8°C for 48 hr. These initial bloods were taken 6 months after Camoprime had been stopped. Camoprime was then reinstated, with two tablets being taken at weekly intervals, for 2 weeks. Within 24 hr of the last dose, repeat blood samples were drawn, and again the cells were cultured both immediately and after a 48-hr delay. As shown in Table 2, when the subjects were not on the drug, and their cells were cultured immediately, the over-all frequency of cells with aberrations was 1.25%, a figure which is in good agreement with the far more extensive Japanese control data. The difference between the frequency of cells with aberrations in the delay-in-culture groups of Table 2 (36 of 800) and the no-delay groups (25 of 1200) is significant statistically ($\chi^2 = 9.48$, $df = 1$, $0.001 > p < 0.01$). The difference in frequency of damaged cells between the on-Camoprime samples (39 of 1200) and the not-on-Camoprime samples (22 of 800) was not significant ($\chi^2 = 0.41$, $df = 1$, $0.50 < p < 0.70$). The most impressive finding in this experiment was the appearance among those cells which were cultured after a delay of 48 hr of dicentrics and rings, aberrations which are rarely seen among controls when there is no delay in initiation of culture. For example, among the Japanese, no rings and only one dicentric were seen in 16,035 cells from 174 persons. In the present experiment, eight dicentrics and one ring were seen among the 800 cells cultured after the 48-hr delay.

Table 3 presents an analysis of all Indian cells with aberrations, by age and sex of the subjects. The regression of the proportion of damaged cells on age by decade is nonsignificant ($b_{x,y} = 0.00613 \pm 0.0026$, $t = 2.305$, $0.05 < p < 0.10$). Further, there is no significant male-female difference in the percentage of cells damaged ($\chi^2 = 0.166$, $df = 1$, $0.50 < p < 0.70$), nor is the difference significant if the multibreak, complex cells alone are considered ($\chi^2 = 2.325$, $df = 1$, $0.10 <$

TABLE 3. (continued)

No. cells examined	Females		No. cells examined	Totals	
	No. complex cells	No. cells with aberrations		No. complex cells	No. cells with aberrations
200	0	7	900	4	30
800	3	19	1475	5	33
300	1	18	1000	3	53
100	0	0	300	4	11
200	0	17	600	2	28
100	0	4	600	3	39
0	0	0	0	0	0
1700	4	65	4875	21	194

$p < 0.20$). Thus, age and sex effects apparently do not account for the distribution of the observed damage.

Discussion. The original purpose of these cytogenetic studies of the Yanomama Indians was to obtain data on the frequencies and types of aberrations which were to be found in a group of primitive people who were not known to be exposed to the same environmental mutagens as civilized man. We expected to establish with this information a base line for the frequency of spontaneous aberrations in human cells. Contrary to expectation, and in the absence of any known exposure to agents identified as producing chromosomal damage, the cultures have yielded a higher than expected frequency of damaged cells, and extraordinary chromosome breakage within a few cells, manifested by the presence of highly complex inter- and intrachromosomal exchanges.

It seems clear from the experiments with the controls that much of the increased frequency of cells with isolated aberrations among the Indians is somehow related to culture conditions. The mechanism by which the delay in culture may exert its effect on aberration frequencies is at present unclear, but several factors must be considered. The heparin used as anticoagulant contains 1% benzyl alcohol (toluene) as preservative. The effects of benzenelike compounds on chromosomes are considerable.¹¹ Cold itself may be a factor. The prolonged period in the vacutainers may have resulted in some degree of anoxia, with resultant cellular degeneration. Interestingly, de Duve has demonstrated that, during cell death, proteases and nucleases are released from the lysosomes.¹² This finding may be important in the present context, since it is clear that delay in initiation of culture does result in fewer viable and dividing cells. The effects of these enzymes on chromosomal nucleoproteins may be considerable. Thus, delays in the initiation of cultures of human cells may produce significant interlaboratory differences in aberration frequencies.

Although culture conditions appear to be responsible for the majority of the cells exhibiting moderate chromosomal damage, the cause or causes of the extreme chromosomal damage observed in a few cells is completely unknown. It seems to us unlikely that these multiple, complex rearrangements are artifactual. Some exogenous insult, which may be quite localized, seems the probable cause. Thus, at the present time we are in the midst of cytogenetic studies on a different Indian tribe of this region, handling the blood samples precisely as the

Yanomama bloods were handled. To date, 2800 cells from 28 Piaroa Indians have been examined and no cells with this extensive damage have been seen.

Within the context of present knowledge, two of the more obvious possible etiologic agents are viral or other infectious agents and toxic plant material. With respect to an infectious etiology, it is noteworthy that most of the Indians sampled for this study had been immunized 1 yr earlier with attenuated live-virus measles vaccine with gamma globulin.¹³ Nichols *et al.* have shown that the frequency of single chromatid aberrations is increased during measles vaccine-induced viremia, but not if gamma globulin is simultaneously given.¹⁴ Since there are still some Yanomama villages which have neither been vaccinated nor reached by measles, we hope to be able to study their members in the future. Harnden demonstrated that chromatid-type aberrations in human lymphocytes are increased for months after the administration of yellow fever vaccine.¹⁵ This may be of pertinence since yellow fever is endemic in the Orinoco River basin. While either or both of these infections might have increased the frequency of chromatid-type aberrations, we have no evidence that they would be likely to increase *in vivo* the frequency of the more complex exchanges of the dicentric and ring chromosome types. However, chromosome pulverization has been shown to be induced *in vitro* by Sendai virus during cell fusion, so that a virus effect cannot be excluded.¹⁶

Other infectious agents might be mentioned. In 4 of the 49 Yanomama subjects, microfilariae were found on the slides of the cultured cells. Several microfilariae are endemic in this region, including *W. bancrofti*, which this parasite most closely resembles. Many of these parasites reside in the lymphoid tissue. No direct evidence is yet available, however, to support the hypothesis that microfilariae may be capable of inducing chromosome damage in human cells.

With reference to toxic plant material, the Yanomama Indians are known to use hallucinogenic agents. The mode of administration is by nasal insufflation, and the concentration in the nasal mucosa may be quite high. The preparations of plant material analyzed to date have a high alkaloid content.¹⁷ Since the use of hallucinogens is almost entirely restricted to adult males, the lack of significant age and sex differences mitigates against the hallucinogens as the primary etiologic agents.

The types of aberrations found are compatible with either acute or chronic radiation exposure, though the extent of chromosome breakage in these complex cells has not been reported for *in vivo* irradiated cells. Even in Hiroshima and Nagasaki, where estimated exposure doses are often said to have exceeded 500 rad,¹⁸ no persons have been found to date with such a high degree of breakage in isolated cells. There is no known unusual radiation source in the Orinoco region.

It may be that several factors have combined to produce this extensive chromosome breakage. While these factors may be unique to this area, it is also possible that we have encountered in extreme form the operation of more ubiquitous agents. The somatic consequences of this chromosome damage are not known, nor is it known whether there is comparable damage to germinal tissue. Because of the remoteness of the group, and the small and dispersed nature of its villages, collection of satisfactory data on the possible consequences of the chro-

mosomal damage will be extremely difficult. An effort is underway to clarify further the role of culture conditions in producing chromosomal damage; to extend these observations to neighboring tribes; to establish by repeat studies the reproducibility of the findings; to identify by experimental and epidemiological approaches the responsible agents; and, despite the difficulties, to establish the consequences of this damage.

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