Table 2. Percent concentrations of sweeteners and blends of sweeteners equivalent ($n > 20$, $P < .05$) to selected sucrose concentrations in water and in tea. Abbreviations as in Table 1.

Sweetness equivalent (grams of sucrose per 100 ml)	Sweetener [(g/100 ml) × 10^{-2}]			
	D + SS + S + CC			
	In water			
2	1.10			
4	2.50			
4	1.00	0.20		
8	2.00	0.60		
12	3.00	1.50		
4	0.85	0.15	130	
12	1.60	0.80	400	
4	0.55	0.10	100	3.30
12	1.00	0.50	200	7.50
	In tea			
2	1.25			
4	2.50			
4	1.00	0.20		
8	2.00	0.60		

($P < .05$). By these tests concentrations of sweeteners [(grams per 100 ml) × 10^{-2}] equivalent to 2, 4, 8, and 12 percent sucrose in water and 2, 4, and 8 percent sucrose in tea were determined (Table 2).

For descriptive analysis of the dipeptide solutions and sweetener blends which were equal in sweetness to 4 and 12 percent sucrose, there was a panel of ten members. Analyses of variance (*5*) were applied to these data; Duncan's (*6*) new multiple range test was used to locate differences among means (Table 1).

These studies indicate that aspartylphenylalanine methyl ester has potential as a low-calorie sweetener. Because slightly more dipeptide was required for sweetening tea than water, it is likely that changes will be necessary in applying these findings to different beverages or foods. It is not possible to predict whether the dipeptide or the blends of sweeteners will be more or less effective in sweetening, or more or less acceptable, in foods or beverages other than water and tea. Even in water and tea, the flavor attributes might change significantly if ratios of the sweeteners were varied and if sweetness contributed by the individual components was unequal. As with most sweeteners, the dipeptide was more acceptable and relatively sweeter in low concentrations than in high. Hence, use of blends of sweeteners reduces the amount of each needed for desired sweetness, especially when there is synergism among the compounds. Also, by maintaining low concentrations of sweetening agents, problems of toxicity are less likely.

MARION R. CLONINGER
RUTH E. BALDWIN
Department of Food Science and Nutrition, University of Missouri, Columbia 65201

References and Notes

1. Fermco Lab., Div. of G. D. Searle and Co., Chicago, Ill. 60680.
2. Nestea (0.134 percent), The Nestle Co., Inc., White Plains, N.Y. 10605.
3. B. K. Watt and A. L. Merrill, *Composition of Foods* (USDA Handbook No. 8) (Department of Agriculture, Washington, D.C., 1963), p. 16.
4. M. Friedman, *J. Amer. Stat. Ass.* **32**, 675 (1937).
5. G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, ed. 6, 1967), pp. 260–267.
6. D. B. Duncan, *Biometrics* **11**, 1 (1955).
7. Contribution from the Missouri Agricultural Experiment Station Journal series No. 6006.

29 June 1970; revised 27 July 1970

Diurnal Variation of Spontaneous Uterine Activity in Nonpregnant Primates (Macaca mulatta)

Abstract. *Diurnal variations in the average intrauterine pressure and in the average frequency of contraction were demonstrated in four nonpregnant rhesus monkeys. Uterine activity is lowest between 0400 and 0700 hours and highest between 1400 and 1700 hours. There is statistically significant correlation between uterine activity and diurnal variation in core temperature.*

Variations in the contractile pattern that occur without apparent external cause are a characteristic of the spontaneous activity of uterine muscle. This phenomenon has been observed during observations of excised myometrial strips in vitro (*1*) and during studies of nonpregnant human (*2*) and subhuman primate uteri in vivo (*3*).

We used four nonpregnant rhesus monkeys (*Macaca mulatta*), with documented regular menstrual cycles, to delineate some of the factors that produce these alterations of spontaneous uterine motility. After the animals were anesthetized, an open-minded, fluid-filled catheter was introduced, either transcervically or transmyometrially, into the uterine cavity and secured to the uterus with a silk suture. As soon as the operation was complete, the animal was placed in a restraining chair and allowed to recover from anesthesia. Three to five days after surgery, the catheter was flushed with heparinized saline and connected by way of a pressure transducer to a recording polygraph. Spontaneous alterations in pressure within the uterine cavity were monitored continuously until completion of the observations.

On-line electronic integration of the pressure signal was used to facilitate quantitative evaluation of the changes in motility patterns. This measure of the area under the pressure curve encompasses frequency, amplitude, and duration of contraction, as well as the base line pressure during uterine diastole and permits measurement of total uterine activity in physical units for statistical comparison. Average intrauterine pressure was calculated by dividing the total uterine activity by a selected time interval. Frequency of contraction was expressed as the number of complete contraction and relaxation complexes occurring per minute. A contraction was defined as having occurred when the pressure rose 10 mm-Hg above the preceding base line diastolic pressure and subsequently fell below this arbitrarily selected value.

Three animals were maintained in a controlled environment with constant temperature and alternating 12-hour cycles of light and dark, changing at 0500 and 1700 hours. The fourth animal, M-14, was maintained under natural conditions of light and dark (sunrise approximately 0700 hours; sunset approximately 1700 hours).

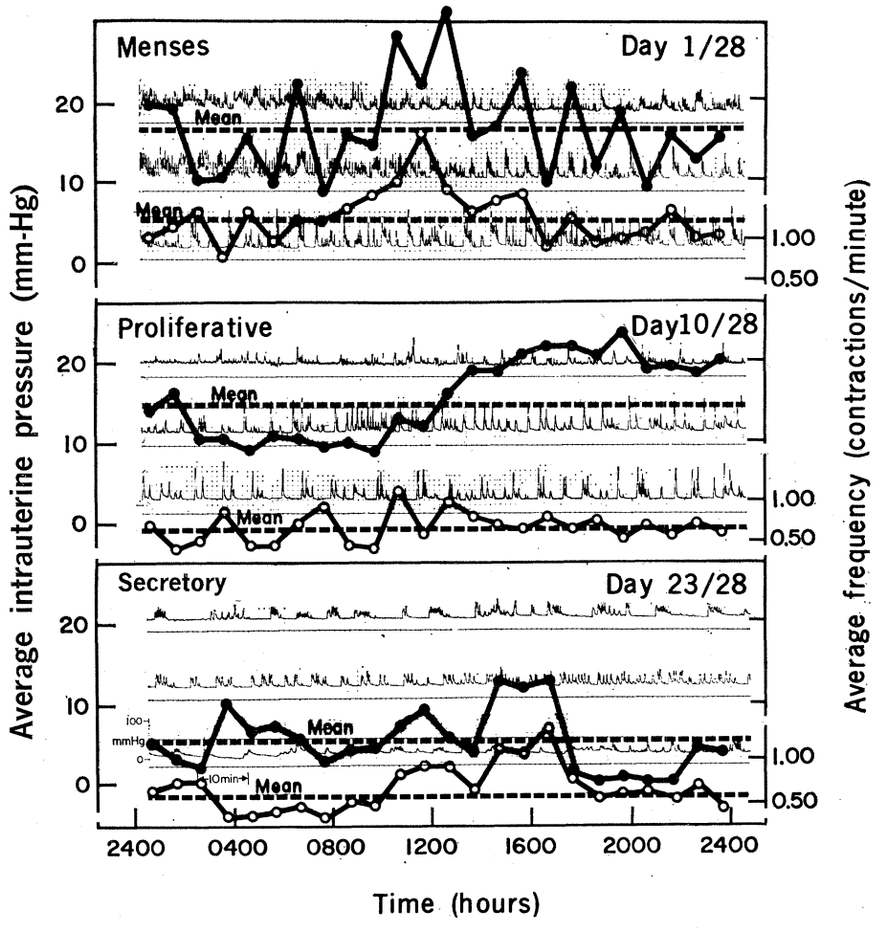
Flushing of the catheter system and checks of the accuracy of the recording system were the only special procedures necessary. Periodically, vaginal smears were obtained for cytological evaluation. In certain instances deep body temperature was continuously recorded by a thermistor placed in the abdominal cavity.

Uterine activity was measured in the four monkeys for a total of 173 days. Figure 1 shows tracings typical of uterine contractile patterns obtained during menstrual, proliferative, and secretory phases of the same menstrual cycle. Tracings obtained between 0600 and 0800, 1200 and 1400, and 2200 and 2400 hours are shown for each cycle phase. The overlay is a graphic presentation of the calculated data. On day 1 of the cycle, mean intrauterine

Fig. 1. Hourly averages of intrauterine pressure and frequency of contraction for 1 day in each of the three phases of a 28-day menstrual cycle are shown against a background of a sample of the polygraph recordings from which they were calculated. The solid points portray values of average intrauterine pressure for each 60-minute interval of recording. The open circles depict corresponding hourly averages of frequency of contraction. The arithmetical means of each parameter for the entire 24-hour period are indicated by the heavy broken horizontal lines. The variation in contraction pattern with cycle phase is evident from the background recordings.

pressure was 17.4 mm-Hg and mean frequency was 1.23 contractions per minute. Uterine activity was characterized by contractions that rose to sharp peaks, often reaching amplitudes over 100 mm-Hg. In the mid-proliferative phase, day 10, contraction frequencies ranged between 0.3 and 1.2 per minute. The contractile pattern included multiple small fluctuations in pressure and sharp, peaked contractions of 80 to 90 mm-Hg. Mean intrauterine pressure was 14.2 mm-Hg. Tracings representative of the secretory phase were obtained on day 23 of the 28-day cycle and had a mean pressure of 5.3 mm-Hg. In this phase of the cycle, uterine activity was characterized by bursts of contractile activity having intensities of 15 to 25 mm-Hg and durations of over 1 minute.

In addition to the distinct differences in configuration of individual contractile complexes and the absolute values of pressure and frequency in different phases of the cycle, there were variations of the hourly averages about the mean for the entire day. To evaluate these differences in daily and hourly average values, we subjected each day of the menstrual cycle of two monkeys to the analysis of variance (*4*). A 28-day menstrual cycle in a monkey (M-46) maintained under controlled conditions of light and dark and a 30-day menstrual cycle of the monkey (M-14)



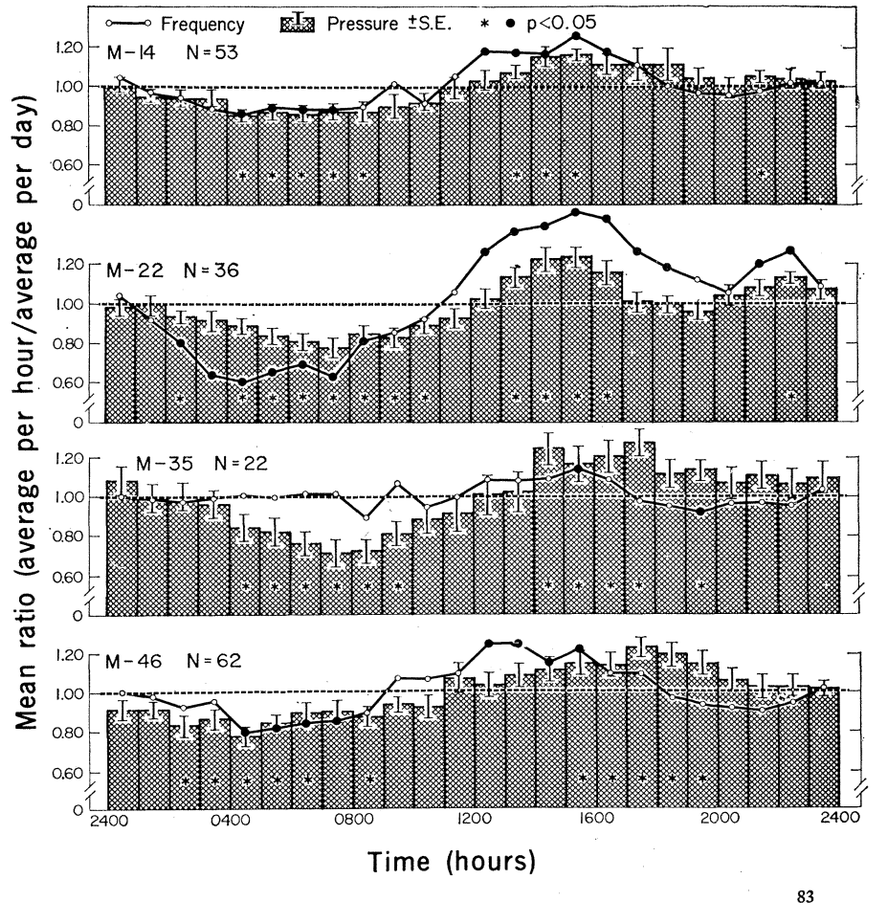


Fig. 2. Diurnal variation of intrauterine pressure and frequency of contraction in four monkeys. The drawings are composites of mean ratios of average pressure and frequency calculated from continuous recordings over periods of 53, 36, 22, and 62 days. The significance of the difference between means for the hour and day was calculated (Student's *t*-test) for each hour, and those that differ significantly at a probability of less than .05 are marked (* ●).

Table 1. Analysis of variance for a 28-day (M-46) and a 30-day (M-14) menstrual cycle.

Term	Degrees of freedom	Average intrauterine pressure (mm-Hg)				Average frequency (contractions per minute)			
		Sum of squares	Mean squares	F ratio	P	Sum of squares	Mean squares	F ratio	P
				Monkey 46					
Days	27	21,336	790	37.61	$<.001$	50.0	1.85	28.92	$<.001$
Hours	23	868	37	1.76	$<.02$	2.7	0.12	1.82	$<.02$
Error	621	13,164	21			29.9	0.06		
Total	671	35,368				92.6			
				Monkey 14					
Days	29	253,627	8,745	61.10	$<.001$	59.09	2.03	22.55	$<.001$
Hours	23	5,244	288	1.59	$<.05$	3.37	0.15	1.62	$<.05$
Error	667	95,465	143			66.58	0.10		
Total	719	354,336				129.04			

maintained under natural conditions of light and dark were used (Table 1).

The average intrauterine pressure and frequency of contraction varied between the two monkeys. This is reflected by the magnitude of difference between the corresponding sum of squares and mean square values. The analyses of variance show that the greater variability is due to differences in pressures and frequencies on various days.

There remains a smaller but significant variability due to differences in pressures and frequencies between hours of the day. For example, monkey M-46 showed a significant hour-to-hour variation in average intrauterine pressure during the 24 hours of the day ($F_{23, 621} = 1.76$, $P < .02$). The F ratios established for variations between the hours of each day for both average pressure (1.76 and 1.59) and average frequency (1.82 and 1.62) were statistically significant.

Figure 2 is a graphic presentation of the hour-to-hour variation in average intrauterine pressure and average frequency of contraction. To minimize the marked differences in magnitude of response between animals and between different phases of the menstrual cycle, the data are summarized as a series of ratios. Individual ratios were established by dividing the average value for each hour of the day by the mean value for the corresponding 24-hour period. The data points on the graph give the hour-to-hour variation of the arithmetical mean of a large number of these individual ratios. A value of 1.00 would indicate that the average for that hour did not differ from the mean value obtained for the entire 24-hour period from midnight to midnight. In each animal, uterine activity, as measured by average intrauterine pressure, was lowest between 0200 and 1000 hours. Significantly lower values of 76 to 88 percent of the daily mean consistently occurred between 0400 and 0700 hours. Subsequently, between 1400 and 1900 hours, average intrauterine pressure progressively increased to maximum values 14 to 23 percent above the mean value for the day. Average frequency of contraction exhibited corresponding variations in three monkeys. In the fourth animal (M-35), statistically significant differences in the frequency of uterine contractions occurred only at 1500 and 1900 hours.

In three animals, deep body temperature and uterine activity were measured simultaneously for periods of 5, 9, and 11 days (Fig. 3). The temperatures recorded at the midpoint of the same hour each day were averaged. In every instance, the correlation coefficients obtained by comparison of average intrauterine pressure and average frequency of contraction with body temperature are statistically significant ($P < .05$). These findings suggest that the diurnal variations of pressure and frequency about the mean values for the day are related to the diurnal variation in body temperature.

Numerous stimuli, both external and internal, may account for these changes. However, their occurrence in all phases of the menstrual cycle would suggest

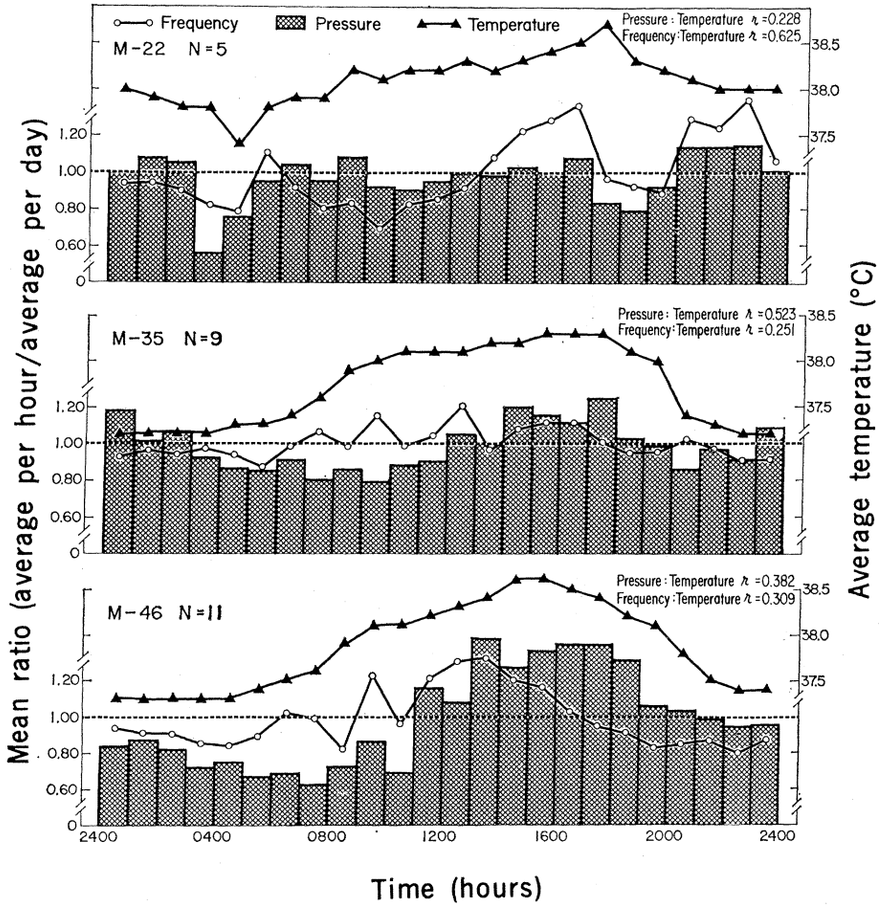


Fig. 3. Correlation between diurnal variation of average core temperature, average intrauterine pressure, and average frequncy of contraction. The drawings are composites of mean ratios of these variables from three monkeys made from continuous recordings lasting 5, 9, and 11 days. The correlation coefficients (r), calculated by comparison of the individual ratios of average intrauterine pressure and average frequency of contraction with body temperature, are shown for each animal.

that they are not due to the alterations in the amounts of estrogen and progesterone during the cycle.

GUY M. HARBERT, JR.
GEORGE W. CORNELL
W. NORMAN THORNTON, JR.
Department of Obstetrics and Gynecology, University of Virginia School of Medicine, Charlottesville 22901

References and Notes

1. J. M. Robson, *J. Physiol.* **79**, 83 (1933).
2. C. H. Hendricks, in *Muscle*, W. M. Paul, E. D. Daniel, C. M. Kay, G. Monckton, Eds. (Pergamon, New York, 1965).
3. S. H. Wax, G. M. Harbert, Jr., W. N. Thornton, Jr., *Obstet. Gynecol.* **29**, 442 (1967).
4. G. G. Simpson, A. Roe, R. C. Lewontin, *Quantitative Zoology* (Harcourt, Brace, New York, 1960).
5. Supported in part by PHS research grant HD-02798.

20 May 1970

Heritable Fragile Site on Chromosome 16: Probable Localization of Haptoglobin Locus in Man

Abstract. *We have found recurrent chromosome breaks at a site (the "fragile site") on the long arm of chromosome 16. This site segregates in simple Mendelian dominant fashion in a large family. The distal portion of the chromosome sometimes shows selective endoreduplication. Preliminary linkage results reveal only 3 recombinants in 33 opportunities for recombination between the fragile site and the alpha locus of haptoglobin, an indication that the* α-Hp *gene is located near this region on chromosome 16.*

Frequent spontaneous breaks may occur at a specific site in a human chromosome (*1–3*). Most reports (*1, 2*) of this phenomenon have involved sporadic cases. However, Lejeune *et al.* (*3*) found a chromosome 2 with a fragile region in a mother and her daughter, and Shaw observed a similar chromosome 2 in an unspecified number of individuals from two unrelated families. We wish to report a large family with simple Mendelian transmission of a fragile region on chromosome 16.

The family was ascertained through an 18-year-old boy, who had recurrent cold urticaria and immunoglobulin A (IgA) deficiency. Chromosome analysis of his lymphocytes (*4*) showed 8 of 33 metaphases figures with an isochromatid break in the long arm of a chromosome 16. The breaks were consistently at the junction of the middle and distal thirds of the long arm (Fig. 1). This region will be designated the "fragile site."

Lymphocyte cultures from the patient showed a variable proportion of cells with breaks at the fragile site. Two cultures labeled with tritiated thymidine (*5*) showed a general increase in both single and isochromatid breaks, but no increase in breaks at the fragile site, compared to unlabeled cultures. A direct preparation of bone marrow chromosomes (*6*) showed 2 of 41 metaphases with an isochromatid break at the fragile site. None of 165 fibroblast metaphases (*7*) showed breaks at that site, and none of the other cases in which fibroblasts were cultured (*2*) revealed a fragile site in fibroblasts. The fragile site on chromosome 16 might conceivably be manifest only in lymphocytes and their precursors in the patient and be related to his IgA deficiency. However, his mother had IgA deficiency with no evidence for the fragile site, whereas his father had normal amounts of IgA and manifested the fragile site.

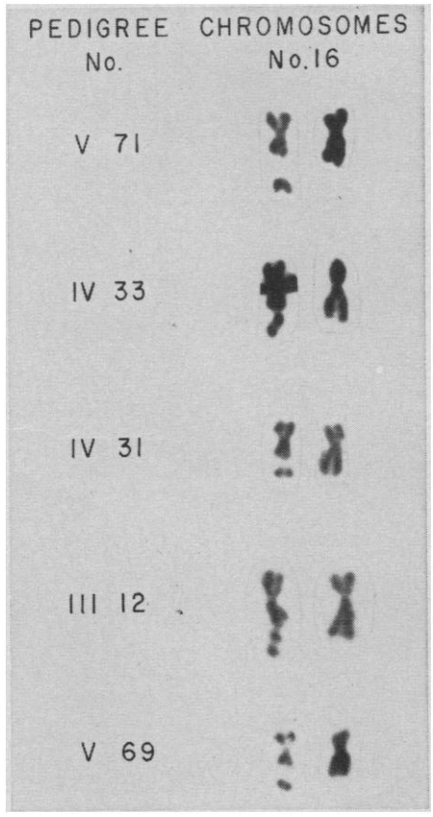


Fig. 1. Composite showing the fragile site on chromosome 16 in the patient and relatives.

Family studies led to the construction of a pedigree containing 238 individuals; lymphocyte cultures from 127 living members have been studied (Fig. 2). The criteria (*8*) for determining the presence or absence of the fragile site in an individual were as follows: (i) a minimum of two metaphases in 60 should show the fragile site; (ii) at least 60 metaphases should be examined before the individual can be said not to have the fragile site; (iii) if one among the first 60 metaphases appears to have the fragile site, another 60 metaphases should be examined, and so on. A minimum of 12 metaphases were also photographed, and karyotypes of at least two metaphases were prepared from each person.

In addition to the father of the patient, 29 other paternal relatives were found to have the fragile site (Figs. 1 and 2). Fifty persons in the family had a parent carrying the fragile site, if it is assumed that III-5 and IV-26 were affected (Fig. 2). Twenty-eight of the 50 had the fragile site; 22 did not. This is not significantly different from the 1:1 ratio expected for simple Mendelian dominant transmission ($\chi^2 = 0.72$; $P > .30$, d.f. = 1). Every person with the fragile site, whose parents were alive, had an affected parent. Unaffected persons had only unaffected children. Thus, the fragile site appears to be fully penetrant.

The cause of the fragility is not known. It is probably due to the particular structure of the fragile site, since there was no generalized increase in chromosome breakage.

Two to 12 copies of the distal fragment (Fig. 3) were observed in some cells from the affected persons. This suggests that the control of replication for the distal portion of this chromosome is not the same as for the remainder of the chromosome. The possibility that the distal fragment undergoes recurrent nondisjunction seems unlikely, since cells were not found with the fragment or the centromeric portion alone, as would be expected with nondisjunction. Lejeune *et al.* and Shaw (*3*) made similar observations in families with a fragile site in chromosome 2. They attributed it to selective endoreduplication, a conclusion in which we concur.

Heritable fragile sites provide an additional tool for use in gene localization. Since the location of these sites can be determined with precision, they