

## Editorial

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### THE IMPORTANCE OF BEING A FRAGILE SITE

The first sighting [1] of a fragile site on a human chromosome was reported in 1965. Since then numerous such sightings have occurred.

The term "fragile site" was coined in 1969 by one of the undersigned (F. H.) during the study [2] of a prolific family with a fragile chromosome 16.

Fragile sites are among the most curious of cytogenetic phenomena. They have not yet been reported in other mammals. They are presumably innocuous. At least most of them are not associated with phenotypic abnormalities. They are never seen in 100% of cells examined. No homozygote has been encountered to date.

In this issue Sutherland provides two valuable articles [3, 4] on fragile sites. The papers are replete with much data and so can hardly be recommended for light bedside reading, although they do contain consequential clues to the "mystery of the fragile sites."

The first paper by Sutherland [3] in this issue is a sequel to his short *Science* article [5] announcing that the expression of fragile sites depends upon the nature of the tissue culture medium. Culture medium 199 reveals fragile sites. Other media veil fragile sites or make their presence much more difficult to demonstrate.

This heuristic observation came not from a lightning bolt of theory, but from one of the main wellsprings of cytogenetics: serendipity. Sutherland's laboratory first used medium 199 for routine diagnostic cytogenetics and detected a number of fragile sites. His laboratory switched in 1973 to another culture medium and ceased seeing fragile sites. Then in 1976 the laboratory astutely began culturing lymphocytes in medium 199 and in another medium to allow contemporaneous comparison of results.

The gist of the story, as it now reads, is that medium 199 is relatively deficient in folic acid; folic acid inhibits the expression of fragile sites, as do folinic acid and thymidine; inhibition can be reversed, however, with a folic acid antagonist, methotrexate.

Serendipity again played a leading role when Sutherland "incidentally" found that the pH of the culture medium had an effect. Elevating the pH increased the proportion of cells expressing *some* fragile sites. Sutherland found further that some fragile sites are relatively indifferent to folic acid concentration.

Sutherland therefore postulates that there are at least three different biochemical classes of fragile sites, as judged by their response to pH, folic acid, and methotrexate.

The second paper [4] by Sutherland in this issue charts the location of the known fragile sites. The sites are on chromosomes 2, 10, 11, 16, 20 and X. Is it meaningful that all these chromosomes with fragile sites are meta- or submetacentric, and that none of the five acrocentrics (13–15, 21, 22) or Y so far is known to have a fragile site?

Chromosome 16 is unique to date in having *two* fragile sites, one on the short (p), and one on the long (q) arm.

What can be learned from the study of fragile sites? The list of what we may learn includes (but is not limited to) the following: (1) Information about *chromosome structure and behavior and the function of certain chromosome bands and subbands*; (2) Genetic information about *disease loci*. The Xq fragile site is, for example, especially intriguing since it is "associated" with a form of mental retardation (Renpenning syndrome) [4–8]. Sutherland neatly sidesteps the decision as to whether the Xq fragile site is simply "associated" with retardation, causes it, or (as seems likely), there is merely close linkage between the Xq fragile site and Renpenning syndrome. The final decision may need to wait until additional families have been studied; (3) *Linkage* information (as with the linkage [2] of the 16q fragile site with haptoglobin); and (4) *Correlation between the genetic and cytologic maps of man*. Most chromosome polymorphisms are situated close to the centromere. The ubiquitous Q- and C-band polymorphisms are all near the centromere on autosomes. The lateral asymmetry types [9, 10] are also usually near the centromere. The newfound Giemsa-11 heteromorphism [11] on chromosome 1 is also near the centromere.

Fragile sites, on the contrary, are more ecumenical in location. Some are near the centromere. Some are interstitial, in and about the "elbow" of chromosome arms. Other fragile sites are situated near the ends of chromosomes. (fig. 9, p. 144).

Fragile sites provide interstitial and distal cytologic markers and so offer the opportunity to correlate the genetic and cytologic maps of our genome, one of the main aims of human genetics today.

Human fragile sites are a potential treasure trove for human cytogeneticists and for all who may have an interest in the genetics of our species.

#### ADDENDUM

##### THE FREQUENCY OF FRAGILE SITES

In October, 1978, while preparing the above editorial, we wrote Dr. Grant Sutherland to inquire about his experience with the frequency of fragile sites.

Since workers considering research on fragile sites may wish to know their frequency, the pertinent parts of Dr. Sutherland's reply are presented here:

. . . for the autosomal sites there is an overall incidence of 9 index cases detected in 4,100 individuals. On the grounds that the autosomal sites are not responsible for any phenotypic change, it is perhaps reasonable to assume that this reflects the general population incidence. The incidence of these specific autosomal sites are as follows: 2q, 10q, 20p, two ascertainment of each; 16p, 16q, 11q, one ascertainment each. I think the only conclusion one can draw from all this is that fragile sites are fairly rare.

The situation with regard to the site on Xq is a little different . . . since they are often present in only a small proportion of the cells, and they were not generally recognized or described in literature apart from the one report by Lubs. . . . We have found 10 index cases with a fragile site on Xq. I am counting any one family as only having one index case, although this isn't strictly correct, since some of the families were identified because they had more than one retarded male. These 10 index cases were among 1,680 individuals karyotyped. The frequency of 10 in 1,680 is of no real

significance, since I have been actively screening populations of retarded males looking for these sites. I haven't really done enough yet to even be able to estimate the frequency of this fragile site even amongst the retarded population.

Apart from the general academic interest (of fragile sites) I believe that it now behooves any laboratory offering a clinical diagnostic cytogenetic service to culture their cells in a medium suitable for the demonstration of fragile sites. I think any cytogenetic reports on retarded males which do not take into account the fact that the retardation could be due to the site on Xq, would have to be regarded as less than adequate.

To all who may be interested in studying fragile sites, it would appear valuable to know that: (1) the frequency of autosomal fragile sites is roughly 1 in 444 (9/4,400) or about 0.2%; and that (2) the frequency of the Xq fragile site in retarded males (or in the general population) is not yet known.

The recommendation of Sutherland to use a medium suitable for the demonstration of fragile sites, especially in studies of retarded males, appears incontrovertible.

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