iron-binding molecules, as well as those that do not, may provide insight into both the bactericidal mechanism of, and the bacterial resistance to, the action of LF.

Both the spectrophotometric and bactericidal assays indicate that on certain microorganisms, LF is capable of exerting a direct bactericidal effect that is contingent upon its chelating properties. This would suggest that LF may be capable of a similar mechanism in mucosal secretions and in neutrophils (*13*). Both the Gram-negative *V. cholerae* and the Gram-positive *S. mutans* were highly susceptible to the bactericidal action of LF at concentrations within the physiological range of many secretions and with numbers of bacteria normally encountered in situ.

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Fragile Sites on Human Chromosomes: Demonstration of Their Dependence on the Type of Tissue Culture Medium

Abstract. *The observation of heritable fragile sites on human chromosomes prepared from lymphocyte cultures has been shown to depend on the type of tissue culture medium in which the lymphocytes are grown. The sites are observed at a much greater frequency when medium 199 is used than when RPMI 1640, Ham's F10, Eagle's (basal), and CMRL 1969 are used. One site on the X chromosome is of clinical significance in that it is a marker for X-linked mental retardation.*

Heritable fragile sites have been described on a number of human chromosomes, including numbers 2, 12, 16, 17, X, and unidentified C-group chromosomes (*1, 2*). When the autosomes are involved these sites are apparently of no clinical significance, but the site on the X is associated with some forms of X-linked mental retardation (*3*).

I recently attempted to reexamine a fragile site on chromosome 2 which had been identified and shown to be familial some years before. Initial studies were puzzling because the fragile site appeared to have vanished. Investigation of this led to the finding that the frequency of lesions at the fragile site depended on the type of tissue culture medium in which the lymphocytes used to produce the chromosomes were cultured. This observation was extended to and confirmed for fragile sites on the X, 10, and 20 (Fig. 1).

Chromosome preparations from lymphocyte cultures grown in parallel in several different commercially available tissue culture media were scored for the presence or absence of a lesion at the fragile site on a number of occasions. The results are shown in Table 1. The frequency of lesions at the fragile site was always much greater when medium 199 was used than with any of the other media. This difference was not so pronounced when the frequency of the lesions was low. When a medium other than 199 was used the frequency of these sites was so low that they would have escaped detection by routine clinical chromosome analysis.

The reasons for these differences in frequency of breakage at the fragile sites according to the type of culture medium are unknown. They may represent either a nonspecific phenomenon related to factors such as *p*H or osmolarity, or be due to a specific chromosome breaking agent present at higher concentrations in medium 199 than the other media used or only present in 199. Delineation of the mechanisms responsible for this effect would be of considerable use.

The findings reported here are of importance in the study of X-linked mental

Table 1. Frequency of observation of fragile sites according to the type of tissue culture medium used. Tissue culture media used were purchased at single strength (except for Eagle's basal medium which was purchased as a × 10 concentrate) from Commonwealth Serum Laboratories, Melbourne, Australia. Lymphocyte cultures consisting of 4 ml of medium, 1 ml of fetal bovine serum, 0.1 ml of phytohemagglutinin, and 0.2 ml of venous blood were harvested according to standard methods after 72 hours of incubation; colchicine was applied for 2 hours and the hypotonic solution used was 0.075*M* KCl. A cell was considered to display evidence of a fragile site if one or both chromatids were broken at the site, if there was chromosome material either in addition or missing which corresponded to the parts of the chromosome on either side of the fragile site, or if a triradial figure was present. Results are expressed as number of cells showing a lesion at the fragile site over the total number of cells examined.

Subject No.	Fragile site	Date studied	Tissue culture medium				
			199	RPMI 1640	Ham's F10	Eagle's (basal)	CMRL 1969
1	2q1	September 1972	23/50				
		May 1976	34/55	3/55			
		June 1976	25/50	0/50	3/50	3/50	2/50
		September 1976	34/50		0/50		
2	10q23	October 1973	36/50				
		May 1976	22/55	1/55			
		June 1976	7/50	0/50	1/50	1/50	0/50
		September 1976	23/50		0/50		
3	20p11	November 1973	33/50				
		May 1976	13/50	5/50	3/50	2/50	
		September 1976	24/50	1/50	0/50	2/50	
4*	20p11	September 1976	22/50		0/50	2/50	
5†	Xq27 or 8	May 1976	8/50	5/50		0/50	
6‡	Xq27 or 8	May 1976	4/50	0/50	0/50	0/50	
		June 1976	5/50	0/50	0/50	0/50	

*Sibling of subject No. 3. †Male. ‡Mother of subject No. 5.

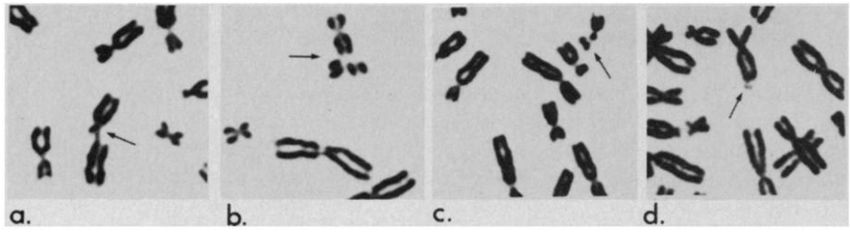

Fig. 1. Different appearances of lesions (arrowed) at fragile sites. (a) Breakage of a single chromatid at 2q1. (b) Triradial figure caused by duplication of chromatids distal to lesion at 10q23. (c) Unstained region of both chromatids at 20p11. (d) Unstained region of both chromatids at Xq27 or 8.

retardation, which has been estimated to account for one-fifth of males with an IQ in the 30 to 55 range (*4*). Most descriptions of X-linked mental retardation have included chromosome studies, but with few exceptions (*3*) these have shown nothing unusual. The fragile site on Xq, which is directly associated with at least one form of X-linked mental retardation, would probably have been missed if such studies were not carried out using medium 199; consequently, they should be repeated.

This association of the fragile site on Xq with one form of mental retardation could provide a means of prenatal diagnosis if the fragile site can be demonstrated in fibroblast cultures. Most reports of fragile sites do not include studies on fibroblast cultures, although Ferguson-Smith (*1*) found the frequency of lesions at a site at 2q to be much lower in these cultures than in lymphocyte cultures. I have been unable to demonstrate the fragile sites in fibroblast cultures from any of the cases in Table 1 regardless of the type of culture medium used.

Further studies of fragile sites are required to determine whether they all behave similarly, what exactly their phenotypic effects are, their frequency in the population, and their relationship to chromosome structure. Identification of the factors in medium 199 responsible for their induction would greatly facilitate this work.

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Species Turnover Rates on Islands: Dependence on Census Interval

Abstract. *Measurements of species turnover in island bird communities demonstrate two trends with increasing census interval* t: *(i) Apparent turnover rates* T *decrease greatly with* t, *and (ii) the coefficient of variation of* T *decreases asymptotically to a constant value. These effects are predicted by a statistical model whose parameters are the immigration and extinction probabilities of each species. Available bird censuses at intervals of decades underestimate turnover rates by about an order of magnitude.*

A fundamental problem of population biology concerns population lifetimes: the species composition of any local community is subject to change with time as local populations of species die out and are reestablished by immigration. While this dynamic structure has received particular attention for island communities, because of the MacArthur-Wilson interpretation that species numbers on islands approach dynamic equilibriums (*1*), similar considerations are also relevant to mainland communities. Thus, censuses of all species present at a given locality at two successive times may reveal "turnover": some species present on the first census may be absent on the second because of local extinctions in the intervening time, and some species absent on the first census may be present on the second because of immigrations. For bird communities on several islands, species turnover rates at equilibrium have been estimated in this way, mainly from comparison of censuses spaced at intervals of several decades (*2–4*).

A major practical problem in turnover studies concerns an "uncertainty principle" associated with the choice of time interval t between censuses. (The problem is analogous to that of an observer who wishes to determine the mean flickering rate of an array of light bulbs flickering at different individual rates, and who faces different types of difficulties depending on whether his frequency of observation exceeds or is less than the flickering frequencies.) The longer t is, the more cases of turnover (either immigrations or extinctions) will be observed, hence the lower will be the coefficient of variation of the apparent turnover rate T. However, with increasing t, T will underestimate the true turnover rate by an increasing factor because a species may repeatedly become extinct and then immigrate (or vice versa) in the time between censuses (the so-called in-and-out effect). This report has four purposes: (i) to illustrate the magnitude of this effect in island bird studies, (ii) to present a simplified statistical theory, (iii) to test the theory against observations, and (iv) to indicate refinements that should be incorporated in a second-generation theory.

Jones and Diamond have obtained annual censuses of breeding land bird populations on each of the California Channel Islands for the past 4 years and have studied results of similar censuses on islands elsewhere in the world (*5*, *6*). Table 1 lists the land bird species that bred on the Farne Islands off Great Britain in each of 29 consecutive years from 1946 to 1974. On these small islands (total area, 32 ha), which are protected as a bird sanctuary and owned by the National Trust, a resident warden and many visiting ornithologists determine the number of nesting pairs of each species each year; the chance that a breeding species would be overlooked is negligible. Of the 16 land bird species that bred on the Farnes in at least one of these 29 years, only four species bred in every year, and only two species "turned over" (immigrated or went extinct) only once. The remaining ten species turned over repeatedly. For instance, meadow pipit bred in the first two census years went extinct by the third year, then successively immigrated and went extinct five times, giving 11 cases of turnover in 29 years. Had only the censuses of 1946 and 1974 been available, one could have deduced only a single instance of turnover for meadow pipit, an extinction. Despite this considerable annual turnover of populations, the number of species breeding on the Farnes remained relatively constant [$\bar{X} = 6$, coefficient of variation (CV) = 0.20]. This finding confirms the basic hypothe-