Chromosomal Breakage in a Rare and Probably Genetically Determined Syndrome of Man

Abstract. *A high frequency of chromosomal breakage and rearrangement has been found in cultured blood cells from six of seven individuals with a rare syndrome characterized by congenital telangiectatic erythema and stunted growth. Only 19 instances of this apparently genetically determined disorder are known, and malignant neoplasia has developed in three.*

Bloom (*1*) first described a clinical syndrome characterized by low birth weight, stunted growth, and a sun-sensitive telangiectatic skin disorder resembling the erythema and facial butterfly lesion of lupus erythematosus. Only 19 individuals with this syndrome are known (*2*). Of 13 families from which information is available, nine are of Jewish ancestry. Of the 16 families involved, three have affected siblings, and in five there is parental consanguinity; taken together, these findings point to a recessive mode of inheritance.

The nucleated cells from the venous blood of seven of the 19 individuals were cultured for 3 days in the presence of phytohemagglutinin (*3*). The chromosomal complement in all seven subjects appeared normal, but in six there was an unusually high frequency (4 to 27 percent) of cells with broken, often rearranged, chromosomes (Fig. 1). Simple chromatid breaks were not increased in frequency. Isochromatid breaks and associated displaced acentric fragments and sister chromatid reunions were common, as was transverse breakage at the centromere which produced two separated telocentric chromosomes. Another very characteristic aberration, present in cultured cells from each of the six individuals with increased breakage, was the equal and symmetrical quadriradial configuration, described previously (*4*). Quadriradial configurations were present in as many as 5 percent of the dividing cells, suggesting a high rate of somatic crossing-over if the behavior of chromosomes in vitro reflects their activity in vivo. More complex abnormal rearrangements, including triradials, asymmetrical quadriradials, and dicentrics were not uncommon. Such chromosomal aberrations have been extremely infrequent in cells from other cultures prepared in our laboratories, except for those from one patient. This was a child (*5*) with congenital anomalies of the radius, hands, hip, heart, and kidneys and an abnormal bone marrow suggestive of the earlier stages of Fanconi's anemia, another genetically determined disorder. Because the high frequency of chromosomal breakage does not seem attributable to any of the usual causes—that is, the action of irradiation, viruses, or certain chemicals—it seems plausible to assume, for the present, that it is associated with the genetic abnormality. Furthermore, it is possibly related to the apparent increase in frequency of malignant neoplasia in persons with this syndrome. Two of those described in the original report (*1*) have died of acute leukemia, one at age 13, the other at 25 (*6*), and in another individual described by Lewis (*7*), a malignant solid tumor has recently developed at age 32.

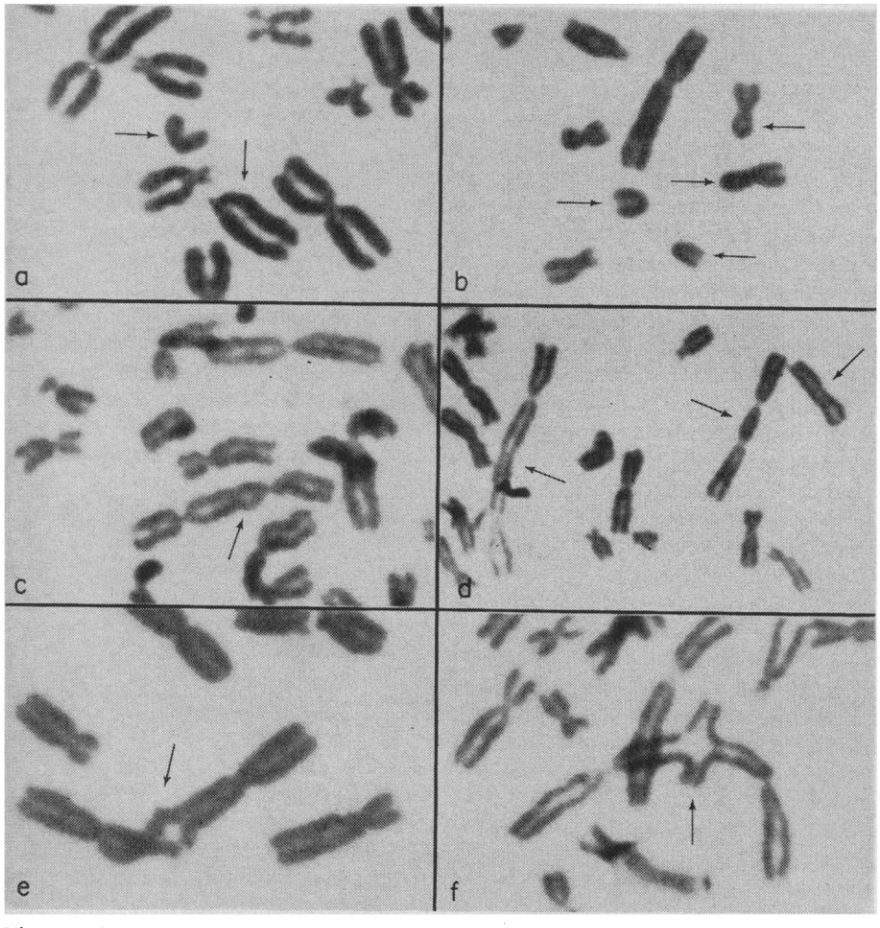

Fig. 1. Six cells from three individuals, showing chromosomal breakage and rearrangement. (*a*) Transverse breakage near centromere; (*b*) sister chromatid reunions; (*c*) dicentric chromosome; (*d*) dicentric chromosomes and an acentric piece; (*e*) and (*f*) quadriradial configurations.

This brief report is presented now, first to demonstrate that an increased tendency to chromosomal breakage may be part of a genetically determined disorder, and second, so that it may encourage the reporting of other patients with such a condition, the study of whom might contribute to an understanding of mechanisms of malignant neoplasia.

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References and Notes

1. D. Bloom, *A.M.A. J. Diseases Children* **88**, 754 (1954).
2. ———, *J. Pediat.*, in press.
3. Experiments conducted in laboratories at the Rockefeller Institute and at Cornell University Medical College.
4. J. German, *Science* **144**, 298 (1964).
5. R. Archibald, M. Erlandson, J. German, in preparation.
6. A. Sawitsky, D. Bloom, J. German, in preparation.
7. B. Lewis, *A.M.A. Arch. Dermatol.* **75**, 772 (1957).

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Mutagenic Effects of Hydroxylamine in vivo

Abstract. *Hydroxylamine can induce any one of the four types of transition mutations in bacteriophage S13 if the mutagen is added directly to agar plates seeded with phage and bacterial indicators. The phage can also be mutated by treating the host cell with hydroxylamine before infecting it with phage. These effects of hydroxylamine in vivo contrast with the mutagenic effect in vitro, which seems to be exclusively on cytosine.*

Hydroxylamine has a highly specific mutagenic effect on bacteriophage treated in vitro. From a study of the induction of forward and reverse mutations, it has been inferred that in the single-stranded DNA of phage S13, only one of the four bases is altered in mutations induced by treatment of the free phage with hydroxylamine (*1*). By comparing that inference with data on the chemical reactivity of hydroxylamine (*2*, *3*), it was concluded that it is cytosine that is altered, being replaced after replication by thymine. This result is consistent with mutation experiments on double-stranded DNA (*2*, *4*), although in double-stranded DNA the mutagenic effect cannot be restricted to cytosine, but must ultimately involve both members of the guanine-cytosine base pair. In S13, it was concluded that the mutagenic specificity of hydroxylamine for cytosine was at least 500 times greater than for adenine, guanine, or thymine, in sharp contrast with the effects of two other frequently used mutagens, nitrous acid and the alkylating agent, ethyl methanesulfonate, each of which appeared to mutate all four bases (*1*).

In this report we discuss the mutagenic effect of hydroxylamine on S13 when the treatment is in vivo—that is, when the host cell is treated either during or before infection. Under these conditions, hydroxylamine causes the mutation of all four bases almost indiscriminately.

A set of reference mutations that includes all four types of transitions was studied (Table 1). The base change for each mutation was previously inferred from the effects of a variety of chemical mutagens (*1*, *5*), and reasons have been given (*1*) for the belief that each mutation involves only the one particular base change indicated. The mutants have been described (*1*) except *su*HT9, which is a hydroxylamine-induced mutant that is able to infect both *Escherichia coli* C and *Shigella dysenteriae* Y6R, but which can produce progeny only in the *Shigella* strain. It is shown in Table 1 that all the mutations were induced when hydroxylamine was added directly to the overlay agar together with the parental phage and bacterial indicators. The identities of the mutants were verified by picking at least three mutant plaques of each type and replating on hydroxylamine-free plates. Of all the mutations shown in Table 1, only $h_i1 \longrightarrow h+$ can be induced in vitro; therefore it is clear that hydroxylamine is less specific in its mutagenic action in vivo, regardless of what base changes are assumed in Table 1.

The initial concentration of hydroxylamine was $2 \times 10^{-2}M$, but the rate of diffusion into the 35 ml (approximately) of bottom-layer agar is not known. The mutations must have occurred within 1 hour after plating because mutant plaques were already clearly visible after only 4 hours of incubation at 37°C. The effect of adding from 10 to 80 μmole of hydroxylamine was tested on the mutation $h_i1 \longrightarrow h^+$. The optimum amount of hydroxylamine was between 40 and 60 μmole. Above 60 μmole, the bacterial lawn would not develop well.

The plates contained free phage, infected cells, and uninfected cells, and any of the three could have participated in the mutagenic process. It seems that a mutagenic effect can occur without a direct interaction of the hydroxylamine with the phage because we were able to induce phage mutations by infecting cells that had previously been treated with hydroxylamine but which had been separated from it by centrifugation before infection. This is shown in Fig. 1 for an experiment in which the cells were both grown and treated in broth. The hydroxylamine concentration ($2.5 \times 10^{-2}M$) used for the treatment was nearly the same as the initial concentration in the overlay agar in the plate experiments. We do not know whether some hydroxylamine might become bound to the cells and thus not be removed by centrifugation. We also do not know why there was a large decrease in the number of mutants after 30 minutes of the treatment.

The host, *E. coli* C, used in this experiment is normally nonpermissive for growth of the parent phage, *su*HT9. Two other mutations, $h^+ \longrightarrow h_i1$ and hH43$\longrightarrow h^+$, were also tested for inducibility by prior treatment of *E. coli* C with hydroxylamine, and in these

Table 1. Induction of the four types of transition mutations by addition of hydroxylamine (HA) directly to the agar plates. The plates (*1*) were incubated at 37°C within 10 minutes after the overlay agar containing phage and indicator bacteria were added. Hydroxylamine hydrochloride (Fisher) was made up as a 1*M* solution in 0.2*M* sodium phosphate buffer, *p*H 6.0, and was stored frozen at −10°C. Similar stored stocks were used in previous in vitro experiments (*1*). Mixed bacterial indicators had to be used for the host-range mutants (*1*). The *p*H of the plates was measured with pHydrion papers (Micro Essential Laboratory). With *E. coli* C in the overlay agar, the *p*H changed from 6.3 at the time the plates were seeded, to approximately 6.0 after 6 hours of incubation at 37°C, and to approximately 5.5 after overnight growth.

Mutation			Amount of parental phage plated	No. of mutant phage plaques appearing on a plate	
Phage		Base change*		No HA	50 μmole HA†
Parent	Mutant				
h^+	$\longrightarrow h_i1$	G $\longrightarrow$ A	4×10^7	20	450
h_i1	$\longrightarrow h_i1$	A $\longrightarrow$ G	1×10^8	8	100
h_i1	$\longrightarrow h^+$	C $\longrightarrow$ T	1×10^8	8	90
hH43	$\longrightarrow h^+$	T $\longrightarrow$ C	1×10^8	1	110
hH76	$\longrightarrow h^+$	T $\longrightarrow$ C	1×10^8	2	80
*su*HT9	$\longrightarrow su^+$	T $\longrightarrow$ C	1×10^8	6	≧300
*su*HT9	$\longrightarrow su^+$	T $\longrightarrow$ C	2×10^7	1	100

* The abbreviations are: G, guanine; A, adenine; C, cytosine; T, thymine. † Added to 2.5 ml of overlay agar.