However we think that, immediately after the period when the volcano emitted large amounts of ash rich in fluoride, a certain quantity of this halogen had accumulated in the leaves, but was subsequently washed away by the rain. At the time of sampling, all accumulated fluoride had disappeared.

Two days after the 5/5/70 eruption of Hekla in Iceland, the concentration of fluoride in grass was 4300 µgF/g (3) at sites where the depth of ash was 10mm. Forty days after the beginning of the eruption, the fluoride concentration was less than 30 µgF/g. This decrease was due in part to heavy rain fall during that time. In the first days after the eruption of Hekla, the concentration of water soluble fluoride in the ash varied between 1400 and 2000 µgF/g.

Remarks

The accumulation of fluoride in the vegetation of volcanic regions must be affected by the high proportion of SO_2 present in the atmosphere. In fact, studies carried out in fumigation chambers have shown that in many cases, leaves accumulated less fluoride from air polluted by SO_2 + HF than leaves polluted solely by HF (6). This decrease in accumulation of fluoride could be the result of closure of the stomata of leaves caused by the presence of SO_2 (7). Thus, we are led to believe that the accumulation of fluoride found in the samples of vegetation growing near Mount Etna must be less than that which would have occurred if the volcano had not emitted large quantities of SO_2 along with the fluoride.

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1977

CYTOGENETIC EFFECTS OF HYDROGEN FLUORIDE GAS ON MAIZE

by

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SUMMARY: Maize seedlings of the genotype C^I Sh Wx were fumigated with hydrogen fluoride gas (HF) continuously for 4, 6, 8 and 10 days. Microspore mitosis of the treated plants indicated the presence of fragments and bridges suggesting the occurrence of the phenomenon of breakage-fusion-bridge cycle of McClintock. This phenomenon was later confirmed by the production of endosperm mosaicisms. The period of fumigation was clearly related to the extent of the area resulting from the B-F-B cycle. Recombination values were estimated from F_2 data for the regions C-sh and sh-wx. There was a significant increase in the frequency of crossing over for region I with maximum increase being for the 4 days duration. The recombination value for region II showed no significant deviation from the control. These findings indicate that HF in addition to being a mutagenic agent is also able to reduce crossing over in certain chromosome segments.

Hydrogen fluoride gas (HF), as an air pollutant, has been clearly shown to be a mutagenic agent (1 - 7). In addition to its mutagenicity, HF was reported by Adams (8), and Ledbetter et al (9) to be a cumulative phytotoxicant. The fumigation of tomato plants and maize seedlings with HF in concentrations below those needed to cause visible injury induced permanent chromosomal changes (1 - 7). Recently, Jagiello and Lin (10) showed that the treatment of mouse, sheep, and cow oocytes, in vitro, with different concentrations of NaF produced meiotic abnormalities similar to those reported by Mohamed, Applegate and Smith in onion root-tip chromosomes (5). These findings supported the suggestions of Muller (11) that some toxic substances which occur as air pollutants, such as HF, may give rise to chemical reactions that result in the formation of mutagens.

In view of the cytological results obtained in maize microsporocytes after fumigation of seedlings with HF (3), the objective of the pre-

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sent studies has been to follow such cytological abnormalities in the post-meiotic mitotic division of the microspores in the treated plants, meiosis in F_1 microspores and to determine the effect of HF treatment on recombination values.

Materials and Methods

Maize kernels of the genotypes C^I Sh Wx and C sh wx were obtained from the Maize Genetics Cooperation. In all of the studies, the recessive genes, including C, which is recessive to C^I, were carried by the seed producing plants. The kernels from both genotypes were germinated in the greenhouse in polyethylene pots containing a horticultural soil mixture.

The procedure of fumigation and treatment was described previously (2). The concentration of the fluoride gas was kept close to 3 µg/m^3. The fumigation of the C^I Sh Wx seedlings was run for 10 days; the first treated plants were removed after 4 days and subsequently plants were removed after 2-day intervals. Control runs were always made simultaneously with treatment runs. After each treatment period the treated and control plants were transplanted into the field. Microsporocyte samples were collected from the fumigated and control plants for microspore mitotic divisions. At pollen shedding, pollen grains were collected separately from each treated and control plant and used to pollinate the recessive seed carrying parent. After the mature harvested F_1 ears were dried and shelled, the kernels were classified according to the different recognizable pheontypes (12 - 14).

A sample of seeds from each of the F_1 populations was planted in the field to produce F_1 plants. Microsporocytes were collected from each plant for meiotic analysis. At the time of sexual maturity the F_1's from treated and control plants were selfed to determine any change in recombination values for the chromosome nine marker genes under investigation: C^I-C (colorless vs. colored aleuron), Sh-sh (nonshrunken vs. shrunken endosperm), and Wx-wx (non-waxy vs. waxy endosperm).

Results and Discussion

A. Meiotic Analysis: Anaphase and telophase studies of the microspore mitosis of the treated plants showed the presence of dicentrics with or without fragments as well as fragments alone (Figs. 1 - 3). The presence of such chromosomal abnormalities may well be explained on the basis of reunion of broken chromatin to form the dicentrics. The presence of heterozygous inversions (3) in the treated plants and the occurrence of crossing over within the inversion loop might explain such findings. Furthermore, the fact that HF affects enzymatic activities in plants (9) might cause a delay in meiotic cycle. According to Rees (15-16) and Darlington and Haque (17), microsporocytes delayed in meiotic activity can exhibit chromosomal breakages. Such breakages may eventually lead to the formation of structural changes and fragmentation. It can be seen from Fig. 1 that the acentric fragment lies in the cytoplasm. This indicated that this fragment was produced either in the first or the second meiotic division and was carried into the telophase II nucleus and later to the post-meiotic division. This would happen if the acentric fragment was close enough to be surrounded by the newly developed nuclear envelope. The mode by which fragments could become included in a telophase II nucleus was explained fully by McClintock (18). Since HF can cause stickiness of chromosomes (3), the acentric fragments may get attached to any chromosome and be included within the nuclear boundry. This is another method by which the acentric fragment might be transmitted to the following generation.

Figures 1-3
First Mitosis in the Microspores

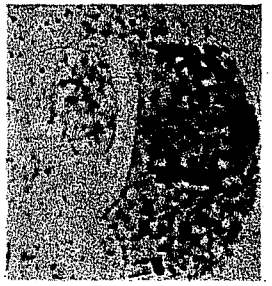

Fig. 1 - Anaphase with a fragment (indicated by arrow).

Fig. 2 - Late anaphase with the fragment lying in the equatorial plane.

Fig. 3 - Telophase showing a broken bridge.

Cytological studies on the F_1 plants microsporocytes confirmed the presence of translocations as well as inversions reported earlier by Mohamed (2).

B. Endosperm Mosaicisms: The cytological basis and behavior for endosperm mosaicisms was provided by McClintock (18 - 19) with the discovery of the B-F-B cycle. In maize endosperm, this phenomenon is recognized by the different patterns that appear, depending upon the endosperm genic markers in use in the experiment.

The data dealing with endosperm mosaicisms derived from the hromatid B-F-B cycle initiated from the breakages produced in either the re-meiotic or meiotic cycle by HF treatment is given in Table 1. Illustra-

Table 1
Frequency of Spotted Areas and Mosaics
in Kernels due to the B-F-B Cycle in Percentage

ment ays	Spots 1	2	3	>3	Small areas	Medium & Large areas	No. Kernels
	27	21	11	15	25	1	411
	11	16	14	40	17	2	597
	14	17	12	27	26	4	167
	9	18	13	22	26	12	161

tive mosaic kernel types are shown in Figures 4- 9. Table 1 shows that the longer the period of fumigation the larger the mosaic area resulting from occurrence of the B-F-B cycle. Such findings agreed with those of Bianchi and Giacchetta (12) in their studies of mutations induced by x-rays in maize. Whereas they reported a linearity with dose, the current studies with HF failed to indicate such an effect, at least after 8 days of continuous fumigation. The regression coefficient was 1. 87 unit/treatment. This value holds true for the three shorter periods of treatment but not for that of ten day duration.

The presence of a high frequency of spots (Table 1) was evident in the shorter periods of treatments. This could be attributed to the delayed effect of the B-F-B cycle in the endosperm tissue in the shorter periods of fumigation. Such delayed reactions have been verified cytologically by Rhoades and Dempsey (20) in maize. On the other hand, Neuffer, Jones and Zuber (21) stated the C^I gene produces few dots (spots) in combinations with C allele. However, in the current studies the number of spots do vary according to the duration of treatments even with the same genic combination.

C. Linkage Intensity : The linkage data was based on F_2 ears in which the different allelic genic markers showed no distortion or significant deviation from the 3:1 ratio. The chi-square test for homogeneity showed that such selected ears from each treatment were homogeneous. Table 2 gives the frequencies of crossing over, using the product method, for the gene markers C-sh-wx. It can be seen from these data that in each treatment for region I (C-sh) there was a significant increase in the frequency of crossing over with the maximum increase being for the 4 days (9. 7%) (Table 3). The increase was not of a high magnitude for the other

Figures 4 - 9
Kernels Illustrating Endosperms Mosaicisms

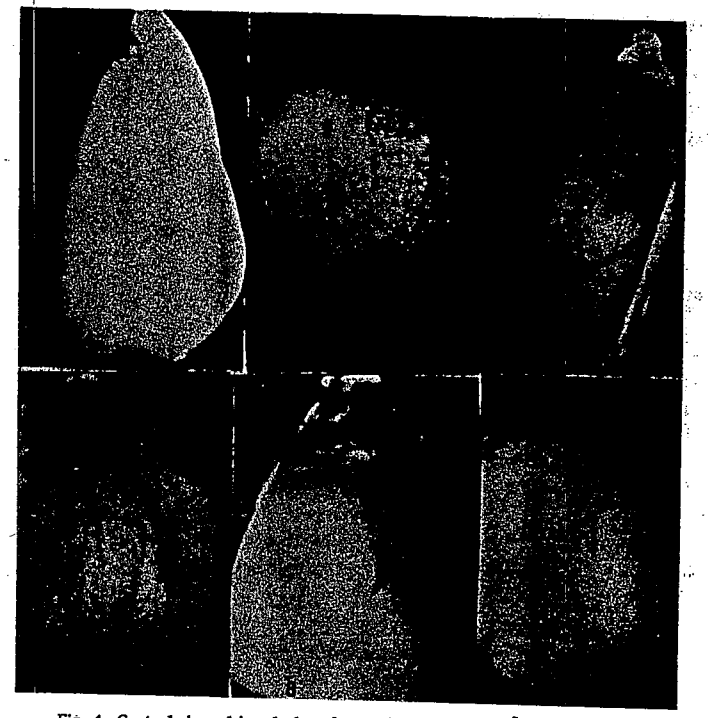

Fig. 4 - Control, kernel is colorless due to the presence of C^I. Figs. 5-7 - Large and medium colored (C). Figs. 8-9 - Small sector or sectors produced by relatively late occurrence of the breaks and the loss of C^I.

treatments, it was nevertheless significant among the different durations of treatments except for the 4 days. The significant reduction in the crossing over frequencies in the 6, 8, and 10 days of treatments, when compared with 4 days fumigation, may be attributed to the induction of minute chromosomal changes or to asynaptic mutants as pointed out by Mohamed (3). On the other hand, the crossover values for region II (sh-wx) did not deviate significantly from the control. According to Mather (22), the total amount of crossing over in the genome is relatively constant. However, a decrease of crossing over in one or more chromosomes due to structural changes would be balanced by an increase in crossing over in other regions. Since the current material exhibits inversions (3), then the reduction in crossing over due to the presence of these heterozygous inversions will be compensated for by the increase in crossing over in another chromosome or

Table 2
Recombination Values, in Percentage, for the Different Treatments

Treatment	Ear No.	Region C-sh	Region sh-wx	Region C-wx	Number of kernels
Control		5.5 ± .8	25.5 ± 1.8	27.0 ± 1.8	851
4 days -	1	13.0 ± 1.9	19.7 ± 2.1	27.5 ± 2.8	379
	2	22.5 ± 3.5	26.5 ± 3.9	30.0 ± 4.1	189
	3	12.7 ± 2.3	21.0 ± 3.0	27.5 ± 3.5	237
	4	12.0 ± 2.6	29.2 ± 4.1	35.5 ± 4.1	178
	5	16.0 ± 1.9	32.0 ± 3.9	31.0 ± 2.8	420
6 days -	1	12.5 ± 1.7	25.5 ± 2.4	25.5 ± 2.4	446
	2	6.5 ± 1.9	22.7 ± 3.7	25.5 ± 3.9	172
	3	8.1 ± 1.6	27.0 ± 3.0	27.0 ± 3.0	313
	4	8.1 ± 1.6	27.8 ± 3.1	25.1 ± 2.9	309
	5	9.1 ± 2.2	24.0 ± 3.8	24.7 ± 3.9	173
	6	7.4 ± 1.3	32.1 ± 2.8	28.8 ± 2.7	427
8 days -	1	10.3 ± 1.8	22.9 ± 2.7	32.0 ± 3.1	338
	2	7.5 ± 1.5	26.7 ± 2.9	31.0 ± 3.2	321
	3	7.1 ± 1.4	22.1 ± 2.6	23.0 ± 2.7	339
10 days -	1	9.0 ± 1.3	32.7 ± 2.7	34.3 ± 2.8	472
	2	5.6 ± 1.2	26.5 ± 2.8	27.1 ± 2.8	361
	3	12.4 ± 1.9	25.9 ± 2.8	30.4 ± 3.0	358
	4	13.4 ± 2.6	27.2 ± 3.7	31.0 ± 4.0	205

* ± Standard Error

region. Similar results were obtained by Redfield (23) in Drosophila. She noticed increases in crossing over in the distal y^2-w^2-spl end of the chromosome when the second and third chromosomes were heterozygous for inversions.

The current data also showed that the region most sensitive to HF is the distal segment involving C-sh. Similarly, Lifschytz (24-25) demonstrated that a rise in temperature increased crossing over in the region car-y^+ in Drosophila melanogaster. He attributed this increase in crossing over to the proximal or intercalated heterochromatin in this region; whether this phenomenon relates to region I, in our present studies, is hard to say. It is well known that DNA replication is somewhat delayed in the heterochromatin region thus reducing the frequency of crossing over under normal conditions. Therefore an alternative explanation for the genetic behavior of the region C-sh may be offered. Cytologically this region is composed of contracted chromatin represented by heavy chromomeres (21) and thus will be delayed under normal conditions in DNA replication. Chromosome breakage in this region followed by reunion of broken ends or merely uncoiling of the chromomeres by HF will increase the physical length of this region leading to an increase in crossing over. This occurs within 4 days of fumigation. On the other hand, with longer periods of HF fumigation the same phenomenon might occur. However, the frequency of crossing over would be expected to be less than that obtained within the 4 days of fumigation, since fumigation of longer duration produces minute deficiencies, cryptic structures or asynapsis between homologous chromosomes (3).

Table 3
Summary of the Pooled F_2 Data for the Recombination Values (r/c), in Percentage for the Different Treatments

Treatment		Region C-sh	Region sh-wx	Region C-wx	Number of kernels
Control	r/c	5.5 ± .8*	25.5 ± 1.8	27.0 ± 1.8	851
4 days	r/c	15.2 ± 1.0	25.7 ± 1.3	30.3 ± 1.5	1403
	diff.	9.7	.18	3.3	
6 days	r/c	8.6 ± .7	26.5 ± 1.2	26.1 ± 1.2	1840
	diff.	3.1	1.0	-.9	
8 days	r/c	8.3 ± .9	23.9 ± 1.6	28.7 ± 1.7	998
	diff.	2.8	-1.6	1.7	
10 days	r/c	10.1 ± .8	28.0 ± 1.4	30.7 ± 1.5	1396
	diff.	4.6	2.5	3.7	

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HYDROFLUOROSIS IN THE FLUORIDATED MILWAUKEE AREA

by

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SUMMARY: The histories of 20 patients with preskeletal fluorosis due to artificially fluoridated water were analyzed. Fourteen (70%) complained of polydipsia. All presented a wide spectrum of symptoms among which polydipsia (70%), general pruritus (55%), headaches (60%) and gastrointestinal symptoms were the most prominent. None of these subjects had been aware, while ill, that fluoride was being added to their drinking water. All made a full recovery when they discontinued the use of fluoridated water for drinking and cooking their food.

In a previous report (1) I presented the history of eight cases of preskeletal poisoning from fluoridated water in the Milwaukee area. Another group of 20 patients interviewed August 1972 provided additional data which are herewith reported. The following are some of the case histories:

Case 4, L.Z., a 60 year old housewife of Cudahy, Wisconsin (fluoridated November 7, 1966), developed a pruritic skin eruption whenever she was bathing. This was followed within a few days by pain in the mid-abdomen, nausea, vomiting and frequent watery stools, by dryness in the throat and marked polydipsia and polyuria. She also had intermittent headaches involving the whole head which gradually became persistent. The patient became listless and her energy waned increasingly. These symptoms continued for about 6 weeks. Then she learned that others were similarly affected from drinking the recently fluoridated Cudahy water. She switched to spring water (0.1 ppm) for drinking and cooking and used melted snow for bathing. Within a week she noted marked improvement and shortly thereafter all the adverse symptoms disappeared.

Case 7, K.D., a 31 year old woman moved at age 18 in 1960 to Milwaukee (fluoridated in August 1953). Within a few days she experienced pains throughout the abdomen which gradually increased in severity; she also had persistent painful urination with frequency and urgency. This condition lasted for nine months when she moved back to her parent's home in Cudahy, which, at that time, was unfluoridated. She made a complete recovery within less than 2 weeks. Two years later she returned to Milwau-

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