TABLE 3

Minerals in Hair of Young Steer after NaF Supplementation
Values in ppm (Dry Matter)

Group of Animals	Period of F⁻ Supplementation	Na	K	Ca	P
Control		1250 ± 82	4720 ± 160	769 ± 18	85 ± 4
Experim.	4 weeks	1880 ± 94	4882 ± 195	1602 ± 24	66 ± 8
Control		1720 ± 86	5040 ± 120	960 ± 15	96 ± 6
Experim.	11 weeks	1422 ± 99	4210 ± 128	972 ± 20	106 ± 10

No data about F⁻ effect on minerals in hair of a bull are available in the literature. Table 3 shows that the normal mineral content increases slightly with age. Sodium and potassium levels in hair had risen in the "fluoridated" group after 4 weeks, but after 11 weeks absorption of these elements appeared to be inhibited by F⁻. Regarding other minerals, F⁻ supplements caused a significant rise in calcium and a slight decrease in phosphorus after 4 weeks. After 11 weeks, there was a trend toward the normal state: The calcium and phosphorus levels were only slightly higher in the hair of the experimental animals than in the controls.

There seemed to be a relationship between the mineral content of blood and that of horny tissues because, after 4 weeks of F⁻ supplementation, a sharp decrease of serum calcium was followed by a rapid rise in calcium of hair. Simultaneously we noted a decrease of phosphorus in hair and an increase in blood serum. This observation supports the view that serum calcium is absorbed by tissues and that a rise in serum phosphorus is due to decreased absorption in tissues. The exchange of minerals between blood and hair may be due to changes in enzymatic pathways caused by high F⁻ supplementation.

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1970

GENETIC EFFECTS OF HYDROGEN FLUORIDE ON DROSOPHILA MELANOGASTER

by

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SUMMARY: Drosophila males were fumigated with gaseous hydrogen fluoride in two series of experiments. In Series I, wild type Oregon-R males were treated by continuous flow of HF for 10 hours. In Series II, dp+/+b males were treated at a steady level of HF for 9 hours. Treated and control males were crossed individually to virgin females of the genotype Pm dp b/Cy. The genetic analyses of the test generations of both series showed that homozygosity for one of the second chromosomes from a treated male resulted in a reduction in viability of the individuals which ranged from subvital to complete lethality. The viability of the controls and the heterozygous sibs was normal. Three individuals of abnormal phenotypes were observed in the segregating generations. None of the crosses conducted to determine the mode of inheritance of these abnormal flies were successful. These limited studies indicate that HF may act as a mutagenic agent.

Every aspect of the biosphere is affected, directly or indirectly, by the quality of the air which envelopes the earth. Adverse effects of air pollution have been demonstrated in natural habitat, agricultural crops, and populations of wild and domestic animals. Gaseous hydrogen fluoride (HF) is one of the most common pollutants. It is probably the most phytotoxic of the halogen compounds. Roholm (1) listed eighteen reports published prior to 1935 on the injury to vegetation and animals from the emission of F⁻ particles or vapor from various industrial plants.

By employing various chemicals, Rapaport (2) was able to induce several of the more common phenocopies and mutants in Drosophila. Among the agents he used which caused specific morphoses, were the ions of heavy metals, inorganic and halogen substituted organic acids. He pointed out that F⁻ induced small melanistic inclusions under the chitin of the larva and imago.

Since the report by Auerbach and Robson (3) on mustard gas as a mutagenic agent, several chemical compounds with varying degrees of mutagenic action have been described and their number is still growing. Muller (4) pointed out that an increasing number of toxic air pollutants may produce their chief damage by injuring the genetic material of the cell which they enter. He also suggested that fluorides may produce this kind of damage indirectly by giving rise to chemical reactions that cause a mutagenic substance to be pro-

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duced. Recently, Mohamed (5) showed that, in tomato plants, HF induced a number of abnormal phenotypes, the same as, or similar to, known mutants.

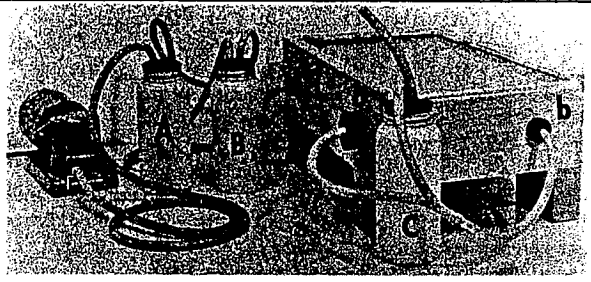
In view of the cytogenetic results obtained in tomato (5, 6) and in maize (7) by Mohamed et al., the objective of the present study has been to test for the mutagenicity of HF on Drosophila.

Materials and Methods

The fumigation cages used in this study (Fig. 1) were modeled somewhat after the population cages developed by Wright and Dobzhansky (8). The overall dimensions were 39 x 25.4 x 10.8 cm with an internal volume of .017m^3. Openings at the ends of adjacent sides allowed admission and escape of the gas.

Fig. 1

Fumigation Cage and Apparatus used in the Present Studies



These openings were plugged with rubber stoppers during the adjustment period. Before the treatment, the cage was lined with parafilm, which is inert to HF and prevents the absorption of F^- ions by the porous wood. A transparent lucite sheet fitted on the top of the cage was sealed with a tile-sealing compound. This lucite sheet permitted observation of the flies during the experiment.

The fumigation apparatus was an electric pump with 3000 rpm used to pump air into the hydrofluoric acid container (A in Fig. 1). Flexible rubber tubing was attached to the pump's pressure outlet and led to the polyethylene container holding the hydrofluoric acid solution. Another piece of rubber tubing from the HF container led to an empty polyethylene container (B in Fig. 1). It served as a trap to prevent the solution from being forced into the fumigation cage. The outlet tubing from container "B" was inserted in a rubber stopper which was fitted snugly into the gas inlet in the cage. The gas outlet was similarly fitted with a rubber stopper and tubing. Each orifice was covered with cheesecloth to prevent the flies from crawling into them. The gas outlet tubing led to a polyethylene container containing a saturated solution of C_aO (C in Fig. 1). It was immersed below the level of the solution. The CaO container was fitted with a short piece of tubing to allow the release of pressure from pumping. The control and treatment cages were identical except that

triple distilled water instead of the acid was used in former container.

Three-day-old D. melanogaster males were subjected to fumigation of gaseous HF prepared from a concentration of 5% hydrofluoric acid. Two series of experiments were conducted. In Series I wild type Oregon-R males were subjected to continuous flow HF for 10 hours. On the other hand, in Series II, males of the genotype dp+/+b were used. In this latter series pumping of HF gas for one hour after which pumping of the gas was stopped. The tubes leading to and from the fumigation cage were clamped off to maintain a constant concentration of HF in the cage until the end of 9 hours. Fumigated males were mated individually to virgin females of the genotype Pm dp b/Cy. This tester stock permitted detection of induced lethal mutations on the second chromosome.

In the F_1 from Series I, five Cy males were selected from each vial and crossed individually with virgin tester females. The Cy progeny of these crosses were sibbed in single pair matings to give the test generation. The Cy pairs were transferred to fresh vials at two day intervals to insure optimal conditions for larval development. In the absence of any viability disturbances, the progeny of these crosses should exhibit the Cy and wild phenotypes in the expected 2:1 ratio. The chi-square test was used to determine whether the deviation observed in the test generation was significant or due to chance alone.

The Cy/Pm method as stated by Wallace and Madden (9) may be used to detect subvital genes, semilethals, lethals, and supervitals. A drawback of strict application of this method is that it cannot determine with certainty which of the test cultures are homozygous for the same chromosome. The utilization of statistical tests may give an indication on this point.

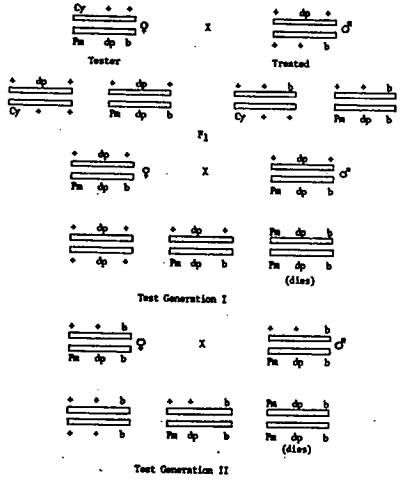
In order to overcome this disadvantage, the Cy/Pm method was modified as in Series II (Pm dp b/Cy X dp +/+b). Since the tester stock carried dp and b on the same second chromosome as the marker gene Pm, the F_1 flies from the treated dp +/+b males and test females which received the Pm chromosome would be homozygous for either b or dp and heterozygous for the other gene, depending on which of the second chromosomes each fly had. Therefore, suitable males and females could be sibbed. A second advantage to this method is that the test generation is produced after two crosses instead of three. Each test culture could be positively identified as being homozygous for either dp or the b chromosome from the treated male (Fig. 2).

Results and Discussion

The three surviving wild type males from Series I were designated by the letters A, B, and C. From the F_1 of these treated males and the tester female, five Cy males were selected from each cross. These five Cy males were given the letters of the treated males and a numeral 1, 2, 3, 4, or 5 to identify them and the subsequent test generations derived from them.

Fig. 2

Series of Crosses Conducted to Detect Recessive Lethals on the Second Chromosome



In Series II, the treated and control males were of the genotype dp+/+b; the ten treated males used for crosses were designated with numerals 1 through 10. Since the F_1 progeny was phenotypically Pm/dp or Pm/b, the test generation derived from each phenotype was given a numeral and the symbol for the homozygous marker gene b or dp.

Table 1 shows the number of flies for each phenotype, the ration of Cy to wild type, and the probability values based on the chi-square test of significance. One male, C-4, died, and the attempted cross failed. One lethal, in test generation A-2, was observed. This culture was maintained for six generations and no wild-type individuals were found.

Among the homozygous progeny, no visible mutations or abnormalities were noted. While this method was not designed to detect visible abnormalities, three individuals of distinctly abnormal morphology were found: 1. A female from the culture A-3 showed a malformed vaginal plate and a grossly abnormal proboscis. It could not be retracted in the normal non-feeding position, but remained extended. This fly was not observed to feed and died within 12 hours. 2. A male from the B-4 test generation exhibited greatly shortened abdominal segments. The terminal segment was truncated and the genitalia were malformed. 3. A male from Series II, test generation 2-dp was less than half of its male sibs in body size. Otherwise it appeared to be morphologically normal. Crosses were attempted with all three abnormal flies, but none was successful. It was, therefore, impossible to determine whether or not these abnormalities were inheritable.

TABLE 1

Comparison Between the F_3 Ratios of the Test Generations of Series I, Based on the Chi-Square Test of Significance

Test generation	Cy	+	Ratio	P value
A-1	113	43	2.6:1	.05
2	122	0	lethal	.0001
3	94	18	5.2:1	.0003
4	111	24	4.6:1	.0003
5	142	60	2.3:1	.3
B-1	90	15	6.0:1	.0003
2	248	42	5.9:1	.0001
3	131	11	11.9:1	.0001
4	102	25	4.8:1	.001
5	284	145	2.0:1	.9
C-1	105	28	3.8:1	.005
2	113	26	4.3:1	.0002
3	157	38	4.1:1	.0001
4*	---	--	-----	-----
5	81	20	4.0:1	.005

*Treated male died.

With respect to the P values (Table 1) all but three test generations A-1, A-5, and B-5, showed a highly significant deviation from the expected 2:1 ratio indicating the ability of HF to induce sublethals in the second chromosome. A possible explanation with regard to A-1 and A-5 is that both represent the same second chromosome of male A which did not undergo any changes affecting viability due to HF treatment. Thus, A-2, A-3 and A-4 may represent the other second chromosome from male A; yet, A-2 contained a lethal. This could be due to gene penetrance.

In the case of the test generations derived from male B, B-5 did not deviate significantly from the expected 2:1 ratio. This may be due to the above stated assumption with regard to A-1 and A-5 test generations.

It can be seen from Table 2 for the F_2 phenotypic classes and ratios of the test generations of Series II, that each of the tested 19 chromosomes carried at least one induced mutation affecting viability.

The relative viability of the homozygotes was compared with the viability of the heterozygotes from the same test generation or with that of the homozygous controls. In each case, the viability of the heterozygous sibs and homozygous controls was assumed to be 100%. The viability values, expressed in percentages, were compared with those of the heterozygotes for Series I and

TABLE 2

Comparison Between the F_2 Ratios of the Test Generations of Series II, Based on the Chi-Square Test of Significance

Test generation	Control Pm	+	Ratio	P value	Treated Pm	+	Ratio	P value
1-b	239	120	2.0:1	.95	346	126	2.8:1	.002
1-dp	205	86	2.3:1	.1	343	126	2.7:1	.005
2-b	367	181	2.0:1	.5	394	120	3.2:1	.0001
2-dp	410	195	2.1:1	.5	272	93	2.9:1	.001
3-b	157	91	1.7:1	.1	552	184	3.0:1	.0001
3-dp	357	181	2.0:1	.5	278	85	3.2:1	.0001
4-b*	---	---	-----	---	519	199	2.6:1	.002
4-dp*	---	---	-----	---	339	115	2.9:1	.0003
5-b	268	133	2.0:1	.5	540	191	2.8:1	.0001
5-dp	265	157	1.7:1	.05	462	128	3.6:1	.0001
6-b	80	47	1.7:1	.2	152	41	3.7:1	.0003
6-dp	92	56	1.6:1	.2	148	42	3.5:1	.002
7-b	389	187	2.0:1	.5	644	162	4.0:1	.0001
7-dp	248	151	1.6:1	.05	392	132	3.0:1	.0001
8-b	409	196	2.0:1	.5	271	71	3.8:1	.0001
8-dp	337	187	1.8:1	.2	630	211	3.0:1	.0001
9-b	124	71	1.7:1	.2	---*	---	-----	-----
9-dp	205	106	1.9:1	.5	43	11	3.9:1	.05
10-b	260	139	1.9:1	.5	332	82	3.9:1	.0001
10-dp	185	101	1.8:1	.3	478	149	3.2:1	.0001

*The control males died. *The treated male died.

TABLE 3

The Viability of the Homozygotes in Series I, with the Viability of the Heterozygotes Assumed to be Equal to 100%

Test generation	Per Cent	Test generation	Per Cent	Test generation	Per Cent
A-1	81	B-1	42	C-1	63
A-2	0	B-2	42	C-2	54
A-3	48	B-3	21	C-3	57
A-4	51	B-4	57	C-4	*
A-5	87	B-5	100	C-5	57

*Treated male died.

TABLE 4

The Viability of the Homozygotes in Series II, with the Viability of the Heterozygotes Assumed to be Equal to 100%

Test Generation	Per Cent	Test Generation	Per Cent
1-b	79	1-dp	81
2-b	70	2-dp	77
3-b	75	3-dp	70
4-b	83	4-dp	76
5-b	79	5-dp	65
6-b	64	6-dp	66
7-b	60	7-dp	76
8-b	62	8-dp	76
9-b	*	9-dp	61
10-b	61	10-dp	72

*Treated male died

$\bar{x}_b$

$\bar{x}_b = 70.3$ $b = \pm 8.9$ S.E.$_{\bar{x}_b} = \pm .99$

$\bar{x}_{dp} = 72.0$ $dp = \pm 6.4$ S.E.$_{\bar{x}_{dp}} = \pm .64$

S.E.$_{\bar{d}} = 1.01$ $t = 1.68$ $P = .10$

II, Tables 3 and 4 respectively. The t-test for the difference in viability between the test generation homozygous for either dp or b gene gave a "t" value of 1.68 with a P value of .10 indicating no significant difference in viability between the means of the two populations.

TABLE 5

Comparative Viability of Homozygotes in Series I and II

Test Generation	Lethal (0-5%)		Semilethal (5-50%)		Subvital (50-100%)		Normal (100%)		Total Chromosomes Tested
	No.	%	No.	%	No.	%	No.	%	
Series I	1	7.1	4	28.5	8	57.2	1	7.1	5
Series II	0	0	0	0	19	100	0	0	19

Table 5 shows the relative viability of the homozygotes for both series. It can be seen from this data that continuous flow of HF (Series I) showed a high frequency of induced lethals and semilethals. On the other hand, in Series II there was a high frequency of subvitals.

It could be concluded, however, that gaseous HF was capable of inducing heritable changes in Drosophila melanogaster. This confirms the results obtained by Mohamed (5) in tomato plants after fumigation with HF. It should also be realized that the data presented here is somewhat limited due to the fact that few chromosomes were tested. Therefore, to arrive at a reliable conclusion, the experiment should be repeated on a large number of individuals to include more chromosomes.

Acknowledgment

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NOTES ON HYDROFLUORIC ACID BURNS

by

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SUMMARY: Intensive treatment with 10% calcium-gluconate injections and with local anesthetics for relief of severe pain are recommended for HF burns. Thorough washing of the lesions with soap and water, debridement of the area and, if necessary, excision of necrotic areas are indicated.

Hydrofluoric acid is the watery solution of HF. It is a colorless, fuming fluid with a boiling point of 19.5°. In the glass industry it is used for etching, polishing or rendering glass opaque; in the metal industry, for processing surfaces. It is also employed in galvanizing and cleaning the outside walls of buildings.

With the expansion of the use of hydrofluoric acid (HF) in industry, the incidence of burns by HF and its vapors has been increasing. In most cases the uncovered face and hands are affected. Even if the hands are protected by loose rubber gloves, the fluid may run inside of the gloves. The eyes are vulnerable to splashes from the acid.

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