On the Radiomimetic Effects of Cupferron and Potassium Cyanide. By B. A. Kihlman. (*From the Institute of Physiological Botany, University of Uppsala, Uppsala, Sweden*)*

Cupferron, the ammonium salt of N-nitrosophenylhydroxylamine, enhances the frequencies of structural chromosome changes produced by x-rays in the root tips of the broad bean, *Vicia faba* (7, 4–6). The enhancement produced at low oxygen pressures (0.3 to 5 per cent O_2 in the gas phase) is particularly striking and appears to be a result of the inhibitory action of cupferron on oxygen consumption (4–6). The enhancing agent itself is not cupferron but oxygen, which, in the presence of the respiratory inhibitor is able to diffuse into the root meristem.

As expected, a similar enhancement of the radiosensitivity at low oxygen pressures is obtained with other respiratory inhibitors (9, 8, 4, 6). In addition to their ability to enhance x-ray sensitivity, the two best studied of these inhibitors, cupferron and potassium cyanide (KCN), have radiomimetic effects; *i.e.*, they are able to produce structural chromosome changes in the absence of radiation (9, 8, 3). Studies on the radiomimetic effects of KCN (3) have shown that the effect is strongest 24 to 36 hours after the treatment, which indicates that KCN does not produce chromosome aberrations in cells which are in the second half of the mitotic cycle during the treatment. The frequencies of aberrations produced by KCN are independent of the temperature during the treatment, but are markedly affected by the concentration of oxygen. Concentrations of oxygen varying between 0 and 100 per cent were tested for their ability to influence the effect of KCN, and it was found that the higher the oxygen concentration, the stronger the radiomimetic effect.

The radiomimetic effect of cupferron appeared to be much weaker than that of KCN (3), and in contrast to the effect of KCN, it was found to be unaffected by variations in the concentration of oxygen. More differences between the radiomimetic effects of cupferron and KCN appear from the experiments described below.

The experimental material in these experiments consisted of lateral roots of the broad bean, *Vicia faba*. The methods of treatment and preparation of slides were the same in these experiments as in those described previously (3).

It appears from Table I that the radiomimetic effect of cupferron is strongly influenced by the pH and the temperature of the treatment solution. The effect increases when the temperature during the treatment is increased or when the pH of the solution is lowered. The toxic effects of cupferron is also increased when the pH is lowered. At pH 5.3, the roots were killed by a 5 hour treatment with a 10^{-3} M solution of cupferron.

The influence of oxygen tension on the radiomimetic effects of cupferron and KCN appears from Table II. In agreement with the results previously obtained (3), the radiomimetic effect of cupferron proved to be independent of oxygen concentration between 1 and 100 per cent oxygen. In an atmosphere of oxygen-free nitrogen, both cupferron and KCN appear to be inactive.

TABLE I

The Influence of Temperature and pH on the Frequencies of Chromosome Aberrations Obtained 30 Hours after 5 Hour Treatments with 10^{-3} M Cupferron. Gasphase: Air

Temperature during treatment	pH of solution	No. of metaphases analysed	Abnormal metaphases	Aberrations/100 cells	
				Isolocus breaks	Exchanges
°C.			per cent		
4–7	6.4	100	0.0	0.0	0.0
4–7	6.4	100	0.0	0.0	0.0
16	6.4	100	11.0	9.0	3.0
16	6.4	100	0.0	0.0	0.0
25	6.4	100	21.0	13.0	11.0
25	6.4	100	18.0	17.0	9.0
20	5.2–5.5	Dead	—	—	—
20	5.2–5.5	"	—	—	—
20	6.3	100	25.0	25.0	11.0
20	6.3	100	17.0	19.0	2.0
20	7.4	100	9.0	7.0	4.0
20	7.4	100	10.0	8.0	5.0

Table III shows that it is possible to obtain a radiomimetic effect of cupferron which is as strong as the strongest effect obtained with KCN (3). In order to get such a strong effect of cupferron, both pH and temperature must be properly adjusted, however.

The results obtained further indicated that the maximum effect of cupferron, like that of KCN,

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TABLE II

The Influence of Oxygen Tension on the Production of Chromosomal Aberrations by Cupferron and Potassium Cyanide at 21°C.

Concentration of cupferron	Concentration of KCN	Concentration of oxygen in the gas phase	pH of solution	Duration of treatment	Duration of recovery	No. of metaphases analysed	Abnormal metaphases	Aberrations/100 cells	
								Isolocus breaks	Exchanges
μM/l	μM/l	per cent		hrs.	hrs.		per cent		
400	—	0.0	6.0	2	26	200	1.0	1.0	0.0
400	—	0.0	6.0	3	26	200	0.0	0.0	0.0
400	—	1.0	6.0	2	26	200	11.5	6.5	6.0
400	—	1.0	6.0	3	26	200	16.5	15.0	4.5
400	—	*Circa* 21 (air)	6.0	3	26	200	9.5	8.5	1.5
400	—	99.7	6.0	2	26	200	9.0	11.5	3.5
400	—	99.7	6.0	3	26	200	9.0	9.0	3.0
—	400	0.0	7.1	1	24	100	0.0	0.0	0.0
—	400	1.0	7.1	1	24	100	1.0	1.0	0.0
—	400	*Circa* 21 (air)	7.1	1	24	200	19.0	23.5	5.0
—	400	" " "	7.1	1	24	100	18.0	21.0	6.0
—	400	99.7	7.1	1	24	100	47.0	50.0	22.0

TABLE III

Comparison between the Magnitude of the Radiomimetic Effects Obtained after Treatments with Cupferron and Potassium Cyanide under Optimal Conditions

Concentration of cupferron	Concentration of KCN	Duration of treatment	Duration of recovery	Oxygen concentration	Temperature during treatment	pH of solution	No. of metaphases analysed	Abnormal metaphases	Aberrations/100 cells	
									Isolocus breaks	Exchanges
μM/l	μM/l	hrs.	hrs.	per cent	°C.			per cent		
1000	—	2	32	*Circa* 21 (air)	25	5.3	200	30.5	24.5	13.0
1000	—	3	32	" " "	25	5.8	200	46.5	38.0	41.5
—	400	1	24	99.7	21	7.1	100	47.0	50.0	22.0
—	1000	1½	30	*Circa* 21 (air)	17	7.0	100	32.0	38.0	13.0

appears between 24 and 36 hours after the treatment.

The experiments have shown that the radiomimetic effects of cupferron and KCN respond differently to factors such as temperature, pH and the concentration of oxygen. Do the differences mean that the mechanisms underlying the radiomimetic effects of the two compounds are different?

The influence of temperature and pH can be explained without postulating that different mechanisms are involved. It seems quite possible that the different response to pH and temperature is a reflection of differences in the ability to penetrate the cell membrane. Small molecules penetrate faster than large ones (1) and it has repeatedly been shown that weak acids penetrate more easily as undissociated molecules (2). Of the two acids corresponding to KCN and cupferron, *viz.*, HCN and N-nitrosophenylhydroxylamine, HCN is to a large extent (*circa* 90 per cent) undissociated even at such high pH values as 8 (10), which may explain why the radiomimetic effect of KCN is not markedly affected by variations of pH between 5.5 and 8. It is also known that the rate of penetration is increased with increasing temperature (11), a fact which would explain why the radiomimetic effect of cupferron is stronger at high temperatures. In the case of the small HCN molecule, however, the penetration may be so fast even at 5°C., that temperature-induced differences in the rate of penetration are of no significance for the radiomimetic effect of ½ to 2 hour treatments with KCN.

The reason why the radiomimetic effects of the two compounds are differently influenced by oxygen pressure is more difficult to explain, and, therefore, this difference could mean that different mechanisms are involved.

Are there any indications of the nature of these mechanisms? According to Lilly and Thoday (9) the chromosomal aberrations observed after treatments with KCN may actually have been produced by hydrogen peroxide, which is believed to accumulate in the cell as a result of the inhibitory action of cyanide on cytochrome oxidase and on peroxide-destroying enzymes. The reasons why this hypothesis is hardly tenable have been discussed in a previous paper (3). The possibility that organic peroxides are involved could not be rejected (3), and since this hypothesis would account for the influence of oxygen, it is still regarded as a possible explanation for the radiomimetic effects of KCN and cupferron. The peroxide hypothesis is less attractive in the case of cupferron, however, since the radiomimetic effect of this compound is independent of oxygen pressure between 1 and 100 per cent oxygen. Another explanation considered in the paper (3) mentioned above, is that the radiomimetic effect is a result of a complex formation between the compounds in question and heavy metals, presumably iron, within the chromosome structure. This hypothesis was partly based on the ability of cupferron and KCN to enhance radiosensitivity in an atmosphere of tank nitrogen. Since this enhancement has been found to be a result of the inhibition of respiration produced by the compounds in question (4–6), the hypothesis is now less attractive. However, both cupferron and KCN enhance the x-ray sensitivity at high oxygen pressures too, and a similar effect has been obtained with ethylenediaminetetraacetic acid (EDTA), which forms complexes with heavy metals but does not inhibit respiration (6, 12). Like cupferron and KCN, EDTA has a radiomimetic effect (6, 12), although this effect is very weak. Therefore, until more evidence is obtained contrary to the idea that chromosome aberrations may arise as a result of a complex formation between these compounds and heavy metals inside the cell nucleus, this hypothesis remains as an alternative to the peroxide hypothesis, at least in the case of cupferron. Other possible explanations are also being considered and the experiments are being continued.

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