C^uDeF, and C^wdEF. Certain less usual combinations are expected: of these *cdeF* has been found, *cDeF*, *CDef*, and *cDEf* are yet to be found.

Anti-*f* is commonly present in anti-*e* and anti-*c* sera.

Anti-*F* is presumed to exist but it will be somewhat difficult to identify.

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[1] Rosenfield, R. E., Vogel, P., Gibbel, Natalie, Sanger, Ruth, and Race, R. R., *Brit. Med. J.*, **1**, 975 (1953).

[2] Race, R. R., and Sanger, Ruth, *Blood Groups in Man*, Blackwell Scientific Publications, Oxford, 1950.

[3] Fisher, R. A., *Ann. Eugen., Lond.*, **6**, 187–201 (1935).

[4] Fisher, R. A., *Ibid.*, **6**, 339–351 (1935).

[5] Finney, D. J., *Ibid.*, **10**, 171–214 (1940).

[6] Cameron, C. (to be published).

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*FURTHER STUDIES ON THE EFFECTS OF CARBON DIOXIDE AND OXYGEN ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA**

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Previous studies have shown that exposure to carbon dioxide during irradiation markedly increases the radiosensitivity of cells.[1–5] It has also been demonstrated that the concentration of oxygen present during irradiation has a profound effect on the radiosensitivity (e. g., Giles and Riley[6]). The experiments to be reported examine the interaction between these two gases and further define the mode of action of carbon dioxide in increasing radiosensitivity.

Material and Methods.—*Tradescantia paludosa* Anderson and Woodson was used in all experiments. Cytological observations of chromosomal

aberration types were made 5 days after irradiation. The principal types of aberrations analyzed at this period are double interchanges (dicentrics and centric rings).

The experimental methods were essentially the same as those described previously.[5] The gases employed were obtained in commercial cylinders and assayed as follows: Nitrogen (Airco), 99.5% N_2 plus 0.5% O_2; Carbon Dioxide (Liquid Carbonic Co.), 99.8% CO_2, 0.05% N_2, 0.009% O_2, 0.07% H_2O. The inflorescences were placed inside $3^1/_2$-liter lucite chambers initially filled with air. In one series of experiments the chambers were first evacuated and then refilled with specific gases at specific pressures. In a second series, the chambers were not evacuated; specific positive pressures of the gas under consideration were superimposed on the atmosphere of air initially present in the chamber.

TABLE 1

FREQUENCIES OF DICENTRICS AND CENTRIC RINGS INDUCED IN TRADESCANTIA MICROSPORES EVACUATED AND THEN X-RAYED IN VACUO OR IN MIXTURES OF AIR AND CARBON DIOXIDE. ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 40 R/MIN. FOR 10 MINUTES

EXPERIMENTAL			CONTROL		
GAS MIXTURE (ATMOSPHERES) CO_2	AIR	DICENTRICS AND RINGS, %	GAS MIXTURE (ATMOSPHERES), AIR	DICENTRICS AND RINGS, %	EXPERIMENTAL AS PER CENT OF CONTROL
0	0	0.9	1	6.4	14
0.33	0	2.0	0	1.7	117[a]
0.33	0	1.9	0	1.8	106[a]
0.33[b]	1	13.4	1	7.3	184
1	0	1.0	0	1.3	77[a]
1[b]	1	23.8	1	7.7	309

[a] Differences of this order of magnitude between experimental and control with aberration frequencies shown here are not statistically significant for the number of chromosomes counted (i.e., 3000).

[b] These preparations were not subjected to preliminary evacuation.

After the desired gas mixture had been established within the chamber, the latter was sealed and was maintained at 25°C. in the dark for a specified period. It was then placed in the x-ray machine alongside an identical chamber containing the dosimeter. Irradiation was performed at room temperature in subdued light. This procedure in each experiment was carried out twice—once with the experimental gas (usually CO_2) and once with the appropriate control gas (usually air). Within 30 minutes after irradiation the chambers were opened and the influorescences returned to air.

Ten to 15 inflorescences were exposed in each chamber. Five days following irradiation as many slides as possible were prepared. Three thousand chromosomes were counted in each control and experimental group. The scoring was performed by one investigator (E. D. K.).

Results.—In an effort to examine the interaction between carbon dioxide and oxygen in conditioning radiosensitivity, the following experiments were carried out. The chambers containing the influorescences were evacuated for $3^1/_2$ minutes and then either maintained in the evacuated state or refilled to a desired pressure with a particular gas. Fifteen minutes after the initial evacuation the chambers containing inflorescences were irradiated for 10 minutes at a constant rate of 40 r/min., after which the chambers were slowly decompressed and the inflorescences returned to air. The results summarized in table 1 permit the following conclusions:

1. Exposure to a vacuum during irradiation markedly decreases the aberration frequency below that encountered in control preparations irradiated in air. This result is in agreement with numerous observations by other workers.[6]

2. Exposure to $^1/_3$ of an atmosphere of carbon dioxide plus 1 atmosphere of air greatly increases the aberration frequency above that of controls irradiated in air.

TABLE 2

FREQUENCIES OF DICENTRICS AND CENTRIC RINGS INDUCED IN TRADESCANTIA MICROSPORES EXPOSED TO X-RAYS IN AIR AND THEN COMPRESSED WITHIN 18 SECONDS WITH CARBON DIOXIDE. ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 200 R/MIN. FOR 2 MINUTES

	GAS MIXTURE (ATMOSPHERES)		DICENTRICS AND RINGS, %	EXPERIMENTAL AS PER CENT OF CONTROL
	CO_2	AIR		
Experimental.....	0.33	1	7.0	96[a]
Control.....	..	1	7.3	..

[a] Differences of this order of magnitude between experimental and control with aberration frequencies shown here are not statistically significant for the number of chromosomes counted (i.e., 3000).

3. Irradiation in the presence of as much as 1 atmosphere of carbon dioxide in the absence of air is equivalent to irradiation in a vacuum.

Thus it appears that carbon dioxide acts synergistically with oxygen in increasing radiosensititivity. But the effect of carbon dioxide on radiosensitivity disappears when oxygen is absent. In short, it appears that carbon dioxide acts by modifying radiochemical reactions that involve oxygen; it affects the "indirect action" of x-rays and not the "direct action" (cf. Sparrow and Rubin[7]).

In a second series of experiments we sought to determine whether carbon dioxide acts at the instant of irradiation or subsequent to radiation. To this end, the inflorescences were first irradiated at 200 r/min. for 2 minutes. Then, within 18 seconds after irradiation, they were compressed with $^1/_3$ of an atmosphere of carbon dioxide. After 60 minutes of exposure to the carbon dioxide, the chamber was decompressed and the inflorescences re-

turned to air. A control group was treated in identical fashion save for omission of the compression with carbon dioxide. Table 2 shows that post-irradiation treatment with carbon dioxide had no detectable effect. Consequently, we conclude that carbon dioxide exerts its action at the time of irradiation. Moreover, since carbon dioxide and oxygen act synergistically, it follows that oxygen acts at the time of irradiation.

Figure 1 records the effects of mixtures of one atmosphere of air plus varying pressures of carbon dioxide. The results of the present study are plotted along with those previously reported.[5] In all these experiments the air-filled tanks and the inflorescences therein were compressed with carbon

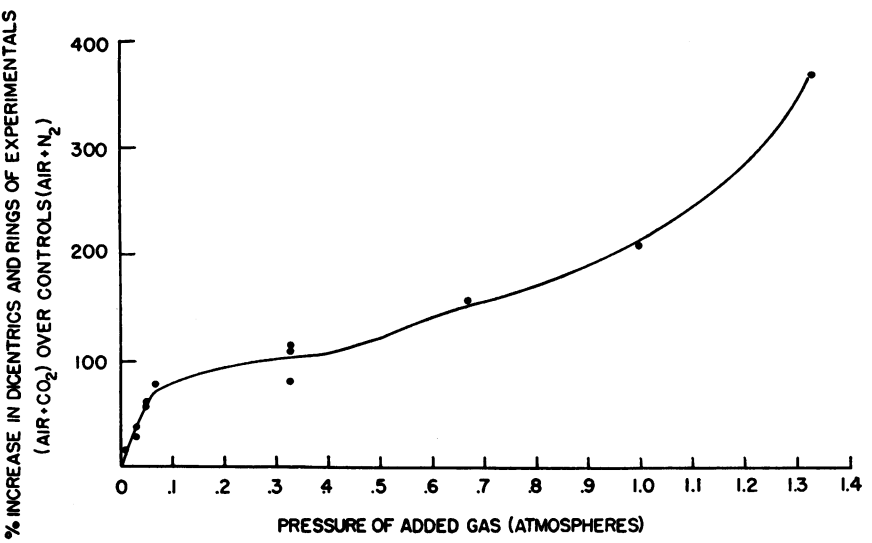

FIGURE 1

Curve showing the effect of mixtures of one atmosphere of air plus varying pressures of carbon dioxide or nitrogen on the incidence of x-ray-induced dicentrics and centric rings in Tradescantia microspores.

dioxide (or nitrogen in the case of controls) for 1 hour prior to irradiation. The inflorescences were then irradiated for 10 minutes at a constant rate of 40 r/min. Within 30 minutes after irradiation the inflorescences were returned to air. As previously noted[5] the results show that the greater the carbon dioxide concentration (up to at least $1^1/_3$ atmospheres of carbon dioxide), the greater the increase in aberration frequency. Moreover, carbon dioxide concentrations of less than 1 per cent of an atmosphere appear to cause a significant increase in aberration frequency.

The relation of carbon dioxide pressure to radiosensitivity in Tradescantia (Fig. 1) differs strikingly from that observed in fern spores and

Drosophila eggs by Zirkle.[2-4] A complex "wave-form" relation between carbon dioxide concentration and radiosensitivity was reported by this investigator: certain concentrations of CO_2 increased radiosensitivity and other concentrations decreased radiosensitivity. Thus, in the case of Drosophila eggs, carbon dioxide tensions between 16 and 21% and above 64% of an atmosphere exerted a protective effect against radiation damage.[3]

Our experiments with Tradescantia reveal no such "wave-form" relation between carbon dioxide concentration and radiosensitivity. To the contrary, radiosensitivity underwent a steady, albeit non-linear increase, as the carbon dioxide concentration increased. Thus carbon dioxide affects chromosome aberrations in Tradescantia in a manner strikingly different from its effects on the radiosensitivity of fern spores and Drosophila eggs. Indeed it appears that carbon dioxide may have widely different influences on different radiobiological systems.

In conclusion we suggest that in Tradescantia carbon dioxide is affecting the "indirect action" of x-radiation. Furthermore, carbon dioxide here appears to affect radiosensitivity only at the time of irradiation. Since carbon dioxide and oxygen act synergistically these observations are consistent with the view that oxygen exerts its action on radiosensitivity at the time of irradiation. Comparison of our data for Tradescantia with Zirkle's data for Drosophila eggs and fern spores leads us to conclude that in different radiobiological systems carbon dioxide may act in very different ways. The information at hand permits no conclusion as to the precise biochemical nature of the carbon dioxide effects.

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[1] Sizova, M. A., *Compt. rend. acad. sci. U. R. S. S.*, **11**, 197 (1936).

[2] Zirkle, R. E., *Am. J. Roentgenology*, **35**, 230–237 (1936).

[3] Zirkle, R. E., *J. Cell. Comp. Physiol.*, **16**, 301 (1940).

[4] Zirkle, R. E., *Ibid.*, **17**, 65 (1941).

[5] King, E. D., and Schneiderman, H. A., PROC. NATL. ACAD. SCI., **38**, 809 (1952).

[6] Giles, N. H., and Riley, H. P., *Ibid.*, **36**, 337 (1950).

[7] Sparrow, A. H., and Rubin, B. A., "Effects of Radiation on Biological Systems," Brookhaven National Laboratories, 97 (T-22) (1951).