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*STUDIES ON THE EFFECT OF CARBON DIOXIDE ON X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA, I. DURATION OF THE PRETREATMENT EFFECT**

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Tradescantia microspores irradiated in one atmosphere of air plus one atmosphere of carbon dioxide show a higher frequency of chromosome aberrations than the air controls.[1–3] This observation has been reported on a variety of organisms.[4, 5] Except that carbon dioxide is an important modifying factor in the induction of chromosome aberrations by radiation, very little is known about the mechanisms involved. The present experiment was undertaken to determine whether the presence of carbon dioxide at the time of irradiation is a necessary condition for the enhancement of chromosome aberrations or whether pretreatment alone is sufficient.

Methods.—The irradiated material consisted of inflorescences from a clone of *Tradescantia paludosa* (Clone 5 of Sax) grown in nutrient solution. The inflorescences were placed in a Lucite chamber fitted with two-way valves and exposed to one atmosphere of air plus one of carbon dioxide for one hour in the dark. The chamber was then evacuated and refilled with air twice. At 1- to 40-minute intervals after the CO_2 treatment, the inflorescences were irradiated with 280 r of X rays (250 KVP, 30 ma, 4.5 mm Al HVL, 280 r/min). Controls were exposed to air alone and whenever possible were irradiated simultaneously with the experimental material. The temperature was maintained at 21–23° C. Throughout this paper the use of the term "CO_2 treatment" indicates a one-hour treatment with 1 atmosphere of air and 1 atmosphere of carbon dioxide.

The inflorescences were fixed four days after the treatment and propriono-carmine squash slides prepared. Those cells which were in metaphase at the time of fixation had been in interphase at the time of irradiation.[6] Microspores in metaphase were scored for dicentrics, centric rings, and interstitial deletions. All of these aberrations require two chromosome breaks for their formation. The slides were coded before scoring and all microscopic observation was made by one person.

Results and Discussion.—The results of two trials of the same experiment carried out four months apart, which were essentially the same, are summarized in Table 1 and Figure 1. The data indicate that treatment of the inflorescences with carbon dioxide before irradiation is effective in increasing the number of chromosome aberrations induced by X rays. The experimental results were tested by chi-square analysis and found to be significantly higher than the controls except for the group where the interval was 40 minutes. In the latter group neither chromosome aberra-

TABLE 1

THE DURATION OF THE CO_2 EFFECT ON CHROMOSOME ABERRATIONS INDUCED BY X-IRRADIATION IN AIR*
(280 r delivered in 1 min)

Time (min) between end of pretreatment and irradiation in air	Exchanges		Interstitial deletions		Total cells
	No.	Per cell	No.	Per cell	
0 (Irradiated in CO_2 + air)	451	0.657	325	0.473	687
1	267	0.608	188	0.428	439
5	225	0.563	172	0.430	400
10	95	0.528	98	0.544	180
15	325	0.518	264	0.420	628
20	331	0.525	261	0.414	631
40	174	0.423	120	0.292	411
Controls (no pretreatment)	361	0.402	292	0.325	898

* One-hour pretreatment with CO_2 and air was followed by an interval in air before irradiation.

tions nor interstitial deletions are significantly different from the controls. The CO_2 effect persists for at least 20 minutes after termination of the pretreatment. The effect of the CO_2 decreases rapidly during the first 10 minutes to a plateau maintained for about 10 minutes and then drops to control level by 40 minutes after the cessation of the treatment (Fig. 1). This initial steep decline and the

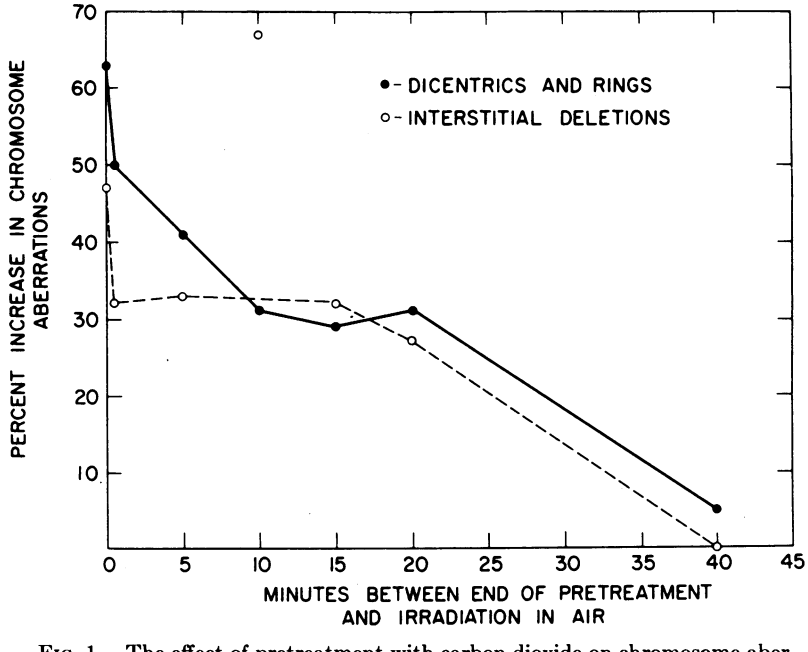

FIG. 1.—The effect of pretreatment with carbon dioxide on chromosome aberrations induced by 280 r of X-rays. The figures on the ordinate express the difference between treated and controls over controls (in per cent) as a function of time in air between the end of the pretreatment and irradiation in air.

subsequent plateau may be indicative of two systems involved in the carbon dioxide effect, but further speculation on this point would be premature.

Recent tests with gamma rays, X rays, 14 Mev neutrons and fission neutrons have indicated that the CO_2 enhancement of chromosome aberrations decreases with an increase in ion density of the radiation used and that significant enhance-

ment obtains only when sparsely ionizing radiations are used.[3] Since chromosome exchanges (dicentrics and rings) are caused mostly by two separate hits with gamma and X rays, but mostly by single hits with neutrons, it was proposed that the CO_2 enhancement applies mostly to two-hit types of aberrations. The hypothesis was advanced that the CO_2 treatment enhanced movement of broken chromosome ends resulting from the irradiation and thus favored new reunions rather than restitution. A significant increase in deletions was also observed indicating that less restitution was taking place. This hypothesis was based on indications that CO_2 decreases the viscosity of protoplasm.[7-11] It was, therefore, reasonable to envisage a greater movement of chromosomes in a medium of lowered viscosity. Furthermore, Northen (1940) has shown that this altered viscosity is not an irreversible state, but that the viscosity of the cell gradually returns to normal after the CO_2 treatment has been terminated. For *Spirogyra* cells, about 15 minutes sufficed for complete recovery of normal viscosity. The present results indicate that the effect of pretreatment with CO_2 on chromosome aberrations like the effect on viscosity is reversible and of relatively short duration.

The results of the present experiment seem to be in agreement with the above hypothesis. In order for the CO_2 pretreatment to be effective irradiation must follow within 20 minutes. This interval may be the period required for the cell to regain its normal physiological state or the period required for the nucleoplasm to return to normal viscosity. Unfortunately, little is known of the viscosity changes in the nucleoplasm. The extensive studies of Kamiya, Allen, and Price[8, 9] concerning the energetics of protoplasmic streaming and viscosity have been performed on the cytoplasm of the slime mold. The fact that CO_2 treatment decreases the viscosity of the cytoplasm of plant cells suggests it may do the same to the nucleoplasm.

Studies of O_2 uptake after CO_2 pretreatment: Several studies have suggested that certain agents affecting respiration of tissues are capable of preventing restitution and thereby keep breaks open to form aberrations over extended periods.[12-14] Since CO_2 is implicated in the respiration of plants and there exist various mechanisms and directions by which excess CO_2 might effect respiration,[15-17] it seemed desirable to determine the oxygen uptake rate of the *Tradescantia* inflorescences at various times subsequent to a carbon dioxide treatment. The oxygen uptake experiments were carefully controlled so as to duplicate the irradiation studies. Two groups of *Tradescantia* inflorescences were placed in a Warburg apparatus to determine the normal oxygen uptake. After the control level had been established, one group was placed in a chamber in 1 atmosphere of air and an additional atmosphere of carbon dioxide was introduced. The experimental chamber was kept in the dark for one hour with the air control. After the pretreatment period the chamber was evacuated twice and refilled with air, just as for the radiation experiments. The flask containing the CO_2 treated inflorescences was removed and placed in the constant temperature bath (21°C) with the control group. A four-minute period was allowed for temperature equilibration and the readings of oxygen uptake, in the light, were taken at regular intervals for one hour. The results are shown in Figure 2.

The results of the oxygen uptake studies indicate that a very dramatic increase in O_2 uptake occurs in the plant tissues either during or within four minutes after

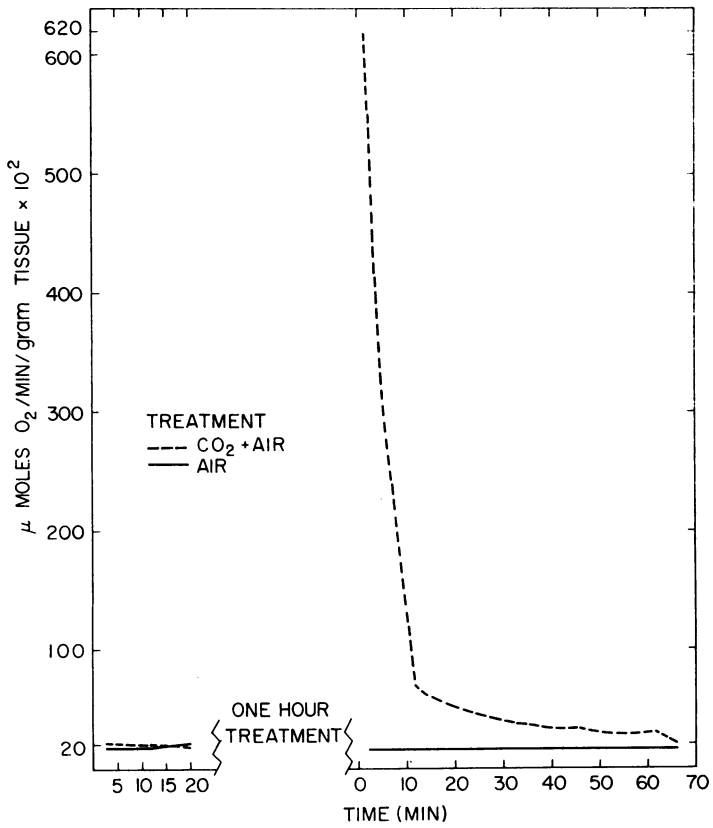

FIG. 2.—Effect of treatment with carbon dioxide on subsequent oxygen uptake in air. The rates were measured before and after the indicated treatment.

they are removed from an environment of CO_2 and air. The oxygen consumption falls very rapidly for about 10 minutes after treatment but remains above the control levels for at least one hour. This can be related to the results of the chromosome aberration studies. The effect of pretreatment with CO_2 on chromosome aberrations is greatest if the irradiation quickly follows the pretreatment. The oxygen uptake during the first four minutes after treatment must be very high, judging from the shape of the curve and the fact that after four minutes it is still 30 times higher than the controls. Chromosome aberrations are 30 per cent higher than the controls during the period 10 to 20 minutes after the pretreatment and the oxygen uptake during this same period is still *twice* that of the controls. The difference between treated and control inflorescences in the oxygen uptake experiments is much greater than the differences observed in the chromosome aberration experiments, especially during the first few minutes. However, the shapes of the curves and the time required for both phenomena to return to control levels are similar.

The carbon dioxide pretreatment is enhancing the radiation-induced chromosome aberrations while the oxygen consumption of the tissues is much higher than the controls. This relationship may be coincidental. It is possible that restitution

of chromosome breaks is not occurring when irradiation follows a CO_2 treatment despite the oxygen uptake rate of the tissues. Perhaps this high O_2 consumption represents the repayment of an oxygen debt incurred during the one-hour CO_2 treatment and that only after the debt has been paid is energy available for chromosome restitution. Also, carbon dioxide treatment may uncouple oxidation from phosphorylation and the high oxygen uptake would reflect an adjustment of the tissues to maintain the level of phosphorylation. Such a mechanism would relate the CO_2 effect to some of the chemicals which have been postulated to inhibit restitution of chromosome breaks.[12-14] In any case, if chromosomes broken by irradiation are not restituting and are moving apart, then when energy subsequently does become available, the probability of forming an aberration is greater than of restitution to the original configuration.

On the other hand, CO_2 may exert its effect entirely by decreasing viscosity, thus enhancing chromosome movement. At the present time, it is difficult to state which portion of an intricate series of cellular changes best accounts for the enhancement of radiation-induced chromosome aberrations caused by pretreatment with carbon dioxide.

Summary.—The pretreatment of *Tradescantia* inflorescences with one atmosphere of CO_2 plus one of air is effective in increasing the number of chromosome aberrations induced by X-rays by about 1.6 times. Separating the CO_2 pretreatment and radiation by increasing periods in air has shown that the CO_2 effect persists for about 20 minutes. After this period the aberration frequency returns to control levels. Following the CO_2 treatment the oxygen uptake rate of the tissues is greatly increased. There is reason to believe that changes in nucleoplasmic viscosity and oxidative metabolism may alter the frequency of chromosome aberrations induced by radiation.

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*BIOLOGICAL CLOCKS IN MEDICINE AND PSYCHIATRY: SHOCK-PHASE HYPOTHESIS**,†

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Biological clocks in medicine and psychiatry we think of as devices of the body that keep time with relative independence of external conditions and events: each in its own units and with varying degrees of accuracy.

What are these clocks? And where are they located? By all odds the best-known example is the ovarian clock, which for several decades of a woman's life determines the time of ovulation, usually at about 28-day intervals.

What other clocks are there? Why aren't they more readily apparent? What makes them run? What part do they play in our lives? Of what interest are they to medicine in general and psychiatry in particular?

Study of these clocks produces new perspectives on the normal and abnormal functioning of various organs of the body, such as peripheral organs, endocrine glands, and the brain.

It is true that normal human beings give little indication of possessing biological clocks—other than the 24-hour clocks of men and women and the 28-day menstrual clocks of women. But many other timed mechanisms are present, as becomes unmistakably clear under certain abnormal conditions.

These clocks manifest their existence through the appearance of one or more of a number of physical and mental symptoms. Let me give a few examples: first, of clocks manifesting themselves through primarily physical symptoms; and second, of others that become evident through primarily mental or emotional symptoms. (I shall in each case present them in order of the lengths of the units in which the clocks measure time, beginning with the shortest intervals we have detected so far.)

CLOCKS THAT MANIFEST THEIR PRESENCE THROUGH PRIMARILY PHYSICAL SYMPTOMS

Evidence for the existence of a clock that measures time in the shortest units (12 hours) is presented in the temperature chart of a 19-year-old girl in Figure 1.[1] Ordinates show body temperature readings taken at frequent intervals throughout the day and night. Peaks of 104.2–105.3°F were reached twice every 24 hours. With ascending temperatures the patient was increasingly ill; once the peak had been passed she felt a remarkable relief from symptoms. Blood and other studies failed to explain the fever.