following: glycine, arginine, lysine, histamine, tryptamine, ethanolamine, gelatin, egg albumin, lysozyme, and gastric mucin at concentrations of 1 to 2 mg/ml (*9*).

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3. The method used was modified from a standard Elson-Morgan (*1*) reaction as follows: Acetonylacetone reagent was made by adding 1 ml of acetonylacetone to 50 ml of 1*N* Na_2CO_3 and was used in place of the standard acetylacetone reagent. To 1-ml aliquots of the materials to be tested was added 1 ml of the acetonylacetone reagent. After mixing, the tubes were capped and placed in a steam bath for 15 minutes, then cooled. Ethanol (7 ml) and Ehrlich reagent (1 ml) (*1*) were added and then mixed. Readings of optical density were taken after 30 minutes at 530 mμ in a Coleman spectrophotometer.
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8. The following two reagents were prepared immediately before use: acetonylacetone dip (50 ml of acetone and 4 ml of acetonylacetone) and Ehrlich dip (50 ml of 9/1 acetone/HCl (vol./vol.) and 0.5 g of dimethylaminobenzaldehyde). The strip was dipped in the former and heated for 5 minutes at 100°C, and then dipped in the latter and dried in the air.
9. This report is a contribution from the Montana Veterinary Research Laboratory (Montana Experiment Station and Livestock Sanitary Board cooperating), Montana State College, Agricultural Experiment Station, paper No. 441, journal series.

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Mutagenic Effect of Oxygen on Barley Seeds

Abstract. Resting barley seeds stored under oxygen at a pressure of 100 lb/in.^{2} for 4 and 6 weeks exhibit a significant number of chromosome aberrations and mutations. The amount of cytogenetic damage increases with length of storage. The frequencies and types of changes are similar to those induced by 500 r to 1000 r of x-rays.

During experiments (*1*) on the relation of oxygen to the aftereffects of x-irradiation in barley seeds, a distinct mutagenic action of oxygen was found. In 1957 resting seeds of barley (*Hordeum vulgare,* variety Himalaya) were stored for 6 weeks under oxygen and under nitrogen; both gases were at 100-lb/in.^{2} pressure. Other seeds were stored in oxygen and in argon at the same pressure for 4 weeks in 1958. Unstored seeds provided the controls. The moisture content of the seeds was maintained at 8 percent throughout the experiments by the presence of calcium chloride in the storage chambers.

Table 1. Frequencies of chromosome aberrations and seedling chlorophyll mutations induced in seeds of barley by oxygen, nitrogen or argon, at 100-lb/in.^{2} pressure.

Treatment	Chromosome aberrations					Seedling mutations		
	No. of cells	Bridges		Fragments		No. of plants	No.	No. per plant
		No.	No. per cell	No.	No. per cell			
				*Experiment 1**				
Oxygen	300	12	0.040†	28	0.093†	468	20	0.04†
Nitrogen	400	2	0.005	14	0.035	468	4	0.008
Control	400	2	0.005	8	0.020	468	2	0.004
				Experiment 2‡				
Oxygen	600	9	0.015	28	0.047†	803	14	0.017†
Argon	600	2	0.003	12	0.020	774	3	0.004
Control	600	3	0.005	9	0.015	761	1	0.001

* 1957 experiment; 6-wk storage period (summary of four replications). † Significant at the 5-percent level. ‡ 1958 experiment; 4-wk storage period (summary of seven replications).

Dicentric bridges and acentric fragments were scored in the shoot tips of M_1 seeds, and seedling chlorophyll mutations were recorded in the M_2 populations. Details of the storage, cytological, and mutation techniques have been published elsewhere (*2*). Because of the method used in gathering the data, it was theoretically possible to obtain numbers larger than 100 percent; however, in the data presented in this paper the numbers are well below 100 percent; therefore differences between means were tested for significance by the method and tables of Davies (*3*) designed for percentage data.

Significant increases in chromosome aberrations and mutations were found following the oxygen treatment in both experiments (Table 1). Neither the argon nor the nitrogen treatments differed significantly from the control treatments. Thus it appears that pressure alone is not mutagenic.

The difference in the number of chromosome aberrations and mutations between the two experiments is considered to be due to the length of time of storage. In the first experiment the barley seeds were under oxygen for 6 weeks, and in the second experiment for only 4 weeks.

Previous reports have recorded either chromosome aberrations or mutations induced by oxygen in biological material. Conger and Fairchild induced chromosome aberrations in *Tradescantia* pollen by high oxygen pressure (*4*). Since the present study was initiated, high oxygen pressure has been reported to induce mutations in *Escherichia coli* (*5*) and chromosome aberrations in seeds of barley (*6*) and *Crepis capillaris* (*7*).

The frequencies of chromosome aberrations and mutations in oxygen-treated seeds are similar to those induced by 500 r and 1000 r of x-rays (*8*); furthermore, the types of changes induced by both mutagens are similar. These results support the postulate of Gerschman *et al.* (*9*) that a common mechanism may be operating in the biological effects of oxygen and x-irradiation.

It is well known that cytogenetic changes occur in aged seeds, although the cause of these changes is not well understood (*10*). The demonstration of the mutagenic action of oxygen in seeds may aid in understanding this process. Over a prolonged storage period, the atmospheric oxygen may directly or indirectly cause the chromosome breaks and mutations that arise in aged seeds. Furthermore, the results described in the present paper are providing an understanding of the relationship of oxygen to post-x-irradiation damage and to the indirect effects of x-rays in seeds.

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References and Notes

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