Effects of High Temperatures on Dry Seeds

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It has recently been reported that when bacterial cells and spores are heated to high temperatures, a high frequency of mutations is induced (19, 20). The treatment is believed to affect the deoxyribonucleic acid (DNA) in that a loss of purines, and concomitantly, a loss of genetic information occurs (5). DNA is rather resistant to heat (18); thus, special methods must be sought if one intends to inflict high frequency of genetic damage to DNA without excessive lethality to the cell caused by high temperatures. It was found that survival at high temperatures and higher mutation frequencies can be obtained by subjecting vegetative cells or spores of bacteria to heat in the dried state under vacuum (20). This is partially due to the fact that without water the proteins are less susceptible to hydrolysis at higher temperatures.

The purpose of this work is to extend these studies to higher plants. Pollen and seeds can be conveniently subjected to drying; only the latter were used in this study.

The effect of heat on seeds and pollen has been the subject of several publications in the past (3, 4, 9–17). It was reported that the survival increases with decreasing moisture content of the heated seeds and that the heating produced delay in germination and other phenotypic as well as genotypic effects.

In the present work it was found that, as in the case of bacteria, on heating dry seeds under vacuum much higher temperatures can be used without lethal effects than in the case of heating at atmospheric pressure. These high temperatures produced pronounced phenotypic effects, both in the physiology and morphology of the plants. In addition, chromosome injuries were observed, some of which may represent genetic damage; this work will be presented at a later date.

Materials & Methods

Seeds. The dormant seeds (except Hordeum vulgare, L., var. Himalaya), used in this work, were obtained from commercial sources. The species were chosen for the highest survival at elevated temperatures and for smallness of seed size (Raphanus sativus, L., Brassica napus, L., Brassica hirta, Moench., Nicotiana rustica, L., Amaranthus tricolor, L., Lycopersicon esculentum, Mill., & Nigella damascena L., var. flor, plen. Miss Jekyll.). Of these, B. napus and R. sativus were chosen for morphological observations: N. damascena lends itself for cytological studies. In addition, H. vulgare, despite larger seeds, was studied for comparison with reported effects of ionizing radiation.

The seeds of H. vulgare were obtained from Dr. Seymour Shapiro, Brookhaven National Laboratory.

Chemicals. Gibberellic acid and kinetin were obtained from Dr. T. Stonier, the Rockefeller Institute. Thiourea was a commercial product. (C.P., Fisher Scientific Co., New York).

Drying. The seeds were subjected to drying for 12 to 72 hours at 35 C in a vacuum oven and then placed in 3 cm diameter glass bulbs (as a layer one seed deep) which were evacuated to 10^-5 to 10^-4 mm Hg (oil diffusion pump) for 3 to 48 hours. In some experiments (see figs) the bulbs, which were still being evacuated, were immersed in a water bath at 55 to 60 C for 3 hours (pre-heating), to improve drying.

Storage. In some experiments, the glass bulbs were sealed under vacuum after the seeds had been dried as indicated above: the seeds were then stored at room temperature for 4 to 70 days before heating to high temperatures as explained below. Note that in the case of bacterial cells this storage before heating greatly improved the survival (20), undoubtedly because of additional water removal. In other experiments the seeds were stored for 14 days (under vacuum or under atmospheric pressure) after heating to high temperatures, before germination.

Heating to High Temperatures. The bulbs with seeds at atmospheric pressure (control) or under vacuum and continuing evacuation as described above (experimental) with or without pre-heating, were heated to 100 to 138 C for 16 minutes, by totally immersing into an oil bath pre-heated to these temperatures. The actual temperature reached by the seeds while heating was measured by a thermocouple inserted into the glass bulb so as to touch the seeds, and connected to an electronic temperature indicator outside of the vacuum system (21). This measurement is considered essential as the data in the older literature often refer to the temperature of the oven and do not permit estimation of the actual temperature of the seeds.

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2 This investigation was supported by research grants No. C-1760 from the National Institutes of Health of the United States Public Health Service, No. G-4337 from the National Science Foundation, and No. E-52 from the American Cancer Society, and by a Senior Research Fellowship SF-241-R from the Public Health Service. An abstract of this work has appeared (1).
3 The plants were grown in the greenhouse of the Rockefeller Institute, New York. The authors are indebted to Dr. Armin C. Braun of the Rockefeller Institute for extending these facilities to them.
Additional measurements have shown that in the system used in the present experiments the surface of the seeds (thermocouple) reaches the temperature of the bath within 1 minute after immersion of the bulb. The smallness of the seeds assures that the embryo in the interior of the seed reaches this temperature shortly afterward.

- Determination of Moisture Content. The moisture content of heated and non-heated seeds was determined in small aliquots of seeds, as the difference between the weight of seeds before and after drying in vacuum for 46 hours at 100°C in a vacuum oven.

- Germination. The main aliquots of heated and non-heated seeds were allowed to germinate on a wet filter paper in covered petri dishes at 23°C or 30°C, either in the dark or under light (two 15-w fluorescent tubes and one 40-watt incandescent bulb, 25 cm from the seeds, 15 hr per 24 hr). In some experiments the filter paper was soaked in 2.5 × 10^-3 M aqueous solution of gibberellic acid or 10^-3 M aqueous solution of kinetin or 3 × 10^-2 M solution of thiourea, as described by Haber et al. (8).

For each species and each heating condition 50 to 180 seeds were used. The germination was scored as successful if the emergence of rootlets and cotyledons alone occurred. One week after germination had started the seedlings were transferred to pots in the green house whenever further study was desirable.

**Results & Discussion**

The loss of moisture (typical examples) of dormant seeds heated in vacuum as described below is represented in Table I.

The results concerning the survival and the delay in germination are represented in figures 1 to 5 which also indicate the particular treatments in each experiment.

As can be seen from figures 1 and 2, the heating in the dry state in vacuum yields surviving seeds at temperatures 20 to 35°C higher than without vacuum. Thus, seeds heated under vacuum survive temperatures as high as 138°C, and such drastic treatment allows one to produce abnormalities not obtainable at lower temperatures.

Dormant seeds of other species tested that survive 120°C (16 min in vacuum) were N. rustica (3% survival), A. tricolor (33%), B. hirta (10–32%), and L. esculentum (11%). Note that high survival temperatures reported in the older literature actually may refer to the ambient temperature and not the seed itself.

Pre-heating to 55 to 60°C and resulting additional drying markedly improves the survival (figs 1 & 2). It is of interest to note that the heat treatment prior to X-ray treatment also reduced final injury (2).

Storage for 24 to 48 hours in a desiccator (vacuum

**Fig. 1.** Germination of dormant seeds of *R. sativus* (radish) in 7 days after heating in vacuum for 16 minutes at temperatures indicated on the abscissa. Curve 1, heating at atmospheric pressure; 2, the same but preceded by pre-heating at 55 to 60°C for 3 hours; 3, heating in vacuum (10^-3–10^-4 mm Hg); 4, the same but preceded by pre-heating as in curve 2. Control seeds, 100% germination. See text for details.

**Fig. 2.** Germination of dormant seeds of *B. napus* (rape) in 8 days after heating. The conditions and the symbols as on figure 1. Control seeds, 98% germination.

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**Table I**

<table>
<thead>
<tr>
<th>Seed Type</th>
<th>Before heating moisture, %</th>
<th>After heating</th>
<th>Final heating, %</th>
<th>Moisture, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. sativus</em></td>
<td>5.3</td>
<td>137</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td><em>B. napus</em></td>
<td>4.8</td>
<td>133</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td><em>N. damascena</em></td>
<td>4.6</td>
<td>120</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td><em>H. vulgare</em></td>
<td>7.2</td>
<td>127</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

* Pre-heating 3 hours, 60°C; final heating 16 minutes at temperatures indicated.
8 mm Hg) prior to high vacuum resulted in a 15 to 18% improvement in survival. However, the same effect can be obtained by replacing the desiccator treatment by a longer (up to 48 hr) evacuation (high vacuum). On the other hand, prolonged storage under vacuum (sealed bulbs), for 2 to 3 weeks before heating, increased the delay in germination and decreased the survival (N. damascena). Storage under vacuum (sealed bulbs) after final heating, for 20 days, had the opposite effect.

The increased resistance to heat upon desiccation under vacuum is probably due to the protection of cell constituent against heat denaturation (hydrolysis) by the removal of water (20) and, possibly, also of oxygen (17).

Among the phenotype changes in the surviving seeds produced by heat the most conspicuous one is the delay in germination (see also 3.9). In the present work delay and inhibition of germination of up to 4 months was studied (N. damascena, fig 3). The addition of gibberellic acid (8) promptly relieved the delay and inhibition (figs 3 & 4) and resulted in a much higher final survival. Kinetin (but not thiourea) (8) also has an effect but to a smaller degree. These studies on a convenient material (N. damascena) may eventually lead to elucidation of the possible sites of injury. For instance, gibberellic acid may stimulate the production of a hormone necessary to overcome the injury or serve as a hormone itself. However, note that the treatment with these substances did not give higher survival temperatures.

It is of interest that all seeds still capable of germination, with or without addition of gibberellic acid or kinetin, completely inhibited the growth of mold, even if germination was delayed up to 4 months.

In addition to delay in germination, the flowering of B. napus was also completely inhibited for at least 2 months. This inhibition could also be relieved by the addition of gibberellic acid.

Figure 5 represents the effect of heating on survival in H. vulgare. Considerable survival can be obtained even at 127 C. The physiological and morphological changes induced by heat and not observed in any of the non-heated controls, included:

A. Germination process: Emergence of the cotyledon and epicotyl before the hypocotyl (B. napus, R. sativus). Elongation of the hypocotyl before the growth of the root [B. napus in 100 % of the seeds; R. sativus in 80 % of the seeds; H. vulgare in 5 %].

The emergence of the cotyledon before the growth of the root (H. vulgare). Doubling of the stem (H. vulgare). Shortening of the stem (H. vulgare). It is to be noted that the latter results are analogous to the effects of radiation (7).

B. Leaves of seedlings: Change in color and the appearance of dark spots (R. sativus); thickening and elongation of the cotyledon, fusion of two leaves, leaves with dissections (notches), abnormal contours, smooth edges, cup-like shape, or larger cotyledons (B. napus).


D. Stem: Fascination (B. napus), formation of additional vegetative buds, and thickening of the stem (R. sativus).

E. Inflorescence: No flowers in 2 months without gibberellic acid; shortening of flowering stalk and reduction in number and size (R. sativus), when gibberellic acid was used.

Some of the possible biochemical injuries by heat have been reported (17).

As mentioned before, some of the morphological

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**Fig. 3.** Delay and inhibition of germination of dormant seeds of N. damascena after heating in conditions as on curve 4 (fig 1), and the effects of $2.5 \times 10^{-3} \text{M}$ gibberellic acid (G) or $10^{-2} \text{M}$ kinetin (K), added at a time indicated by an arrow (for curve G, 121 C, added immediately after heating). C, control (not heated). The temperatures indicated refer to heating in vacuum. See text for details.

**Fig. 4.** The increase in final percentage of germination of heated dormant seeds of N. damascena upon treatment with gibberellic acid (G) and kinetin (K), over the final percentage of germination of seeds heated but not treated with these substances. The values at 118 C represent mean of three experiments. Other conditions as on figure 3.
changes observed after heating to high temperatures may resemble changes induced by ionizing radiation (7); however, the mechanism of injury in these two cases is probably entirely different. The action of radiation may involve the production of free radicals: the action of heat may include coagulation of proteins, breaking of hydrogen bonds, and depurination of nucleic acids (5).

Summary

Previous reports demonstrated that heating of dry bacterial cells and spores to high temperatures in vacuum induced a high frequency of mutations. The treatment was designed to inflict genetic damage to DNA without excessive lethality to the cell (protection of protein by drying in vacuum). To study these effects in higher plants, dormant seeds of R. sativus, B. napus, N. damascena, and H. vulgare were dried and heated (16 min) in vacuum. Survival was obtained at 122 C to 138 C which was up to 35 C higher than without vacuum. Such treatment produced delay and inhibition of germination for more than 4 months (N. damascena); both effects were relieved by gibberellic acid and, to a lesser degree, by kinetin but not by thiourea. Pronounced morphological changes in the resulting plants were also produced. Some of these changes may resemble the effects of ionizing radiation.

Acknowledgments

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Literature Cited