THE BIOELECTRIC POTENTIAL OF SEEDS AS A FUNCTION OF GROWTH AND OF X-RAY DOSAGE

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(with six figures)

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Introduction

Radiation in its passage through matter causes ejection of electrons from atoms on which it impinges. The ejection of electrons may result in both chemical and physical changes in the constituents of cells. These changes are considered to be primarily responsible for the biological effects of radiation.

When a cell is subjected to x-radiation, small islands of chemical change are produced in the region where energy quanta are absorbed. Because radiation penetrates more or less uniformly all parts of small living systems, these islands are produced probably in all the cell parts (membranes, cytoplasm, nuclei and chromosomes). Thus in an organic system that is complex to start with, many different kinds of molecules are destroyed and likewise many new ones come into existence in a chaotic fashion. These changes produce disorganization in either functional or control activities of the cells, or in both. Experimental observations (4) show that generally functional activity is not easily affected by radiation because of the continuous supply of new material through nutrition. Such processes as respiration, metabolism, and enzymatic activity were found to be little influenced by radiation. Dale (4) has shown that only very large doses reduce the activity of enzymes. This reduction can have little importance in tissue unless the sources of enzymes are inactivated.

The exact origin of bioelectric potentials is still open to question although a great deal of work has been done to determine the cause and the seat of the electrical asymmetry responsible for the e.m.f. (5, 6). However, it is recognized that in all biological systems there are a great number of phase boundaries with resulting differences of potential. There undoubtedly are many membrane potentials created by a difference of concentration of the electrolyte on the two sides of each membrane. The islands of ionization produced by the passage of x-rays through biological materials together with the chemical changes caused thereby are bound to affect the bioelectric potentials under any plausible theory of the origin of these potentials.

The object of these experiments is to measure the bioelectric potentials of seeds as a function of growth and of x-ray dosage.

Apparatus and procedure

The electrode system used for measuring the bioelectric potentials was patterned after that of Burr and associates (7). Contact with the speci-
men was made by means of brushes through which flowed 0.1 normal NaCl solution from test tubes in which were immersed Ag and AgCl electrodes.

The silver chloride electrode was prepared by coating a small coil of silver wire electrolytically with silver chloride, a current of 1 ma. being passed through a 10% solution of NaCl for four hours with the silver wire serving as anode and platinum wire as the cathode. Several sets of electrodes were prepared and those which seemed to be the most uniformly coated were used in the experiment.

A somewhat simplified electrode system was used with good results in later experiments. A glass tube about eight inches long and about 3 inch in diameter was bent in the form of an L, and the lower part drawn out and stoppered with about ¼ inch of felt. The metal electrode was introduced into the tube until it touched the felt. The tube was filled with salt solution and sealed at the upper end with wax. The moist felt makes fairly good electrical contact. Care was taken to use two tubes which allow the liquid to pass at the same rate.

In one set of experiments the electrodes were connected by means of shielded wires to a sensitive galvanometer. A potentiometer in series with the galvanometer was used to calibrate the system and to balance out the contact potential. The sensitivity of the system was about 30 microvolts division and was linear over the total range used. The resistance of the path was of the order of two megohms; thus the current drain from the tissue was relatively small. After each measurement the seed was turned through an angle of 180° and a similar measurement taken in the reverse direction. The fact that these two measurements were always in agreement was taken as evidence that the draining of the specimen was small and the circuit was functioning satisfactorily.

In later experiments the electrodes were connected to an electrometer circuit (3) using an FP54 tube with grid resistances ranging from $5 \times 10^7$ to $10^{11}$ ohms. The results were essentially similar.

The x-ray tube was operated at about 60,000 volts. The radiation was filtered through ½ mm. of aluminum. At the location of the seeds the dosage was about 400 r/min.

The seeds were grown in a tray on absorbent cotton, moistened with ordinary tap water. For the measurement of the potential, a seed was placed on a small insulated stand mounted on a revolving base. The brush contacts, which were mounted on adjustable, flexible lever arms, were, in the case of corn, lightly touched to the germinal and micropylar end, i.e., along the longitudinal axis. The galvanometer deflection was noted, then the seed was rotated 180° by turning the base and the measurement of the seed repeated with the reversal of polarity of the e.m.f. to the galvanometer. In the case of corn a large initial deflection was designated the primary potential of the seed and the secondary steady state deflection was designated the steady potential. Nelson and Burr (7) also report a primary and steady state potential of corn seeds. The mean value of these deflec-
tions on the two sides of the zero position was used for the calculation of the bioelectric potential of the individual seed. After a number of preliminary experiments, it was finally decided that a group of 30 specimens was sufficient to give a fair measure of the bioelectric potential at any period of growth of the seeds. This same procedure was followed for the determination of the effects of radiation on the potential and for other studies. The temperature was kept reasonably constant during experimentation. The effects of polarization of the electrodes were negligible and the balancing out of the contact potential was checked at frequent intervals and properly adjusted when necessary.

Results

Bioelectric Potential of Corn Seeds

The corn seeds used for this study were of an inbred variety developed at the University of Florida Agricultural Experiment Station. These seeds are incapable of high yields and were chosen for the particular study of potential versus growth because it was believed that the range of variations of potentials of different specimens for this specific group of seeds would be much lower than the range of variations of potentials of hybrid seeds. This was found to be true as is shown in table I. In most cases, as found by

<table>
<thead>
<tr>
<th>Seed</th>
<th>Average steady potential</th>
<th>Range</th>
<th>Average primary potential</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred (Low yield)</td>
<td>1.65</td>
<td>0.2-5.8</td>
<td>3.70</td>
<td>0.3-12.9</td>
</tr>
<tr>
<td>Hybrid, Oklahoma Silver Mine (High yield)</td>
<td>3.68</td>
<td>0.6-30.0</td>
<td>4.90</td>
<td>1.0-21.0</td>
</tr>
</tbody>
</table>

NELSON and BURR (7), the germinal end of the seed was positive with respect to the micropylar end.

In figure 1, the average steady and the average primary bioelectric potential in millivolts is plotted against the number of hours of growth of the various groups of seeds. It is evident that the increase of the potential is very gradual during the first 30 hours in solution. After 30 hours growth the increase in potential is more pronounced.

It was decided to use a vigorous hybrid type seed for the determination of the effects of x-radiation on the bioelectric potential of corn seeds. Since
the potential of hybrid seeds is higher than that of the inbred variety, it was reasonable to expect a greater variation of the potential with X-ray dose. Tests made on two hybrid varieties, White Dent and Oklahoma Silver Mine, showed that Oklahoma Silver Mine gave higher potentials. It was therefore chosen for study.

![Graph showing bioelectric potential of corn seeds as a function of time of growth.](image)

**Fig. 1.** Bioelectric potential of corn seeds as a function of time of growth.

Comparison between the above data and those observed for inbred seeds (low yielding) shows that the average steady potential of White Dent corn seed for the same time of growth is about twice that of the inbred variety, and the potential of Oklahoma Silver Mine about three to four times that of the inbred variety. These results confirm the conclusions of Nelson and Burr (7) that a higher bioelectric potential indicates a type of seed with a greater yield.

Eight groups of 30 seeds each were planted and six of these groups were
treated with different doses of x-rays ranging from \( \frac{1}{2} \) minute to 10 minutes (i.e., 200 r to 4000 r), the other two groups serving as control. The seeds were irradiated 21 hours after immersion in nutrient solution. The average steady and primary potentials of all seeds were measured at 24 hours of growth (i.e., three hours after irradiation) and again at 48 hours.

Figure 2 shows the variation of the average steady potential with x-ray dose at 24 and 48 hours' growth. The decrease of the potential of the seeds from the normal control group is quite evident. However, even for a dose of 4000 r the bioelectric potential of the seeds is still about one-half that of the mean control which indicates that the dosage given only served to lower the vitality of the seeds but not to kill them. The fact that at 48 hours the potential is higher than at 24 hours is also evidence that the largest dose
was not lethal. This indicates that the growth rate for the irradiated groups approaches the growth rate of the control. Thus, on the basis of Lea's work (4), one would assume that in time the irradiated seeds would have recovered completely. It was not known whether the injury was of a permanent nature or not, since the seeds were not grown to maturity.

![Graph](image)

**Fig. 3.** Length of coleoptile of wheat seeds subjected to various radiation doses as a function of time of growth.

It is noted that a dose of 4000 r seems to lower the bioelectric potential slightly more than does a dose of 800 r. The fact that the curve approximates an exponentially decreasing function is in accordance with the radiation studies of Lea.

At the time of measurement of the 48-hour potentials of the irradiated seeds the sprouts of the various groups of seeds were counted. The length
of the sprouts was not measured. It was noted that the 200 r dose did not affect the sprouting, but as the dose increased the number of sprouts became less.

Two different groups of 30 seeds, small and large, were selected with regard to relative size from a large batch, then weighed and grown for a period of about 47 hours under standard conditions as outlined above. The seeds chosen for this study were of the inbred type used for the growth study presented earlier. The potentials of seeds were measured along the longitudinal axis. No significant change in the bioelectric potential with weight of the seeds was observed.

**Bioelectric potential of wheat seedlings**

The work with wheat was particularly interesting because wheat seedlings at a very early stage of their growth provide easily measurable coleoptiles, the length of which may be taken as an index of the vigor of the
The difference of potential was measured between the tip of the coleoptile and its base. Since the primary and steady potentials were very nearly the same no attempt was made to distinguish between them.

Four groups of seeds have been subjected to various doses of x-rays 12 hours after immersion in the nutrient solution, and the length of the coleoptile, as well as the magnitude of the potential of each seedling, were measured. The series of curves in figure 3 shows the variation with time of growth of the average length of coleoptile for each group for various doses. The curves show that the inhibition of growth of the coleoptile by irradiation takes place very soon after the dose is administered though the effects at first are not very great. The diminution in the length of the coleoptile with dose becomes more pronounced as time goes on; however, even the largest dose (6000 r units) administered under these conditions (after 12 hours in solution) is not lethal since the coleoptile still continues to grow.
This is even more clearly demonstrated in figure 4, showing the variation with time of growth of the average potential of each group subjected to different doses. The heavy doses of radiation seem to inhibit cell activity greatly only at the beginning, but after 72 hours or so the seed seems to overcome, partly at least, the paralyzing effects of the rays and cell activity is resumed with great vigor. Figures 5 and 6 show the effect of radiation on the length of coleoptile and on the potential of the seed at various stages of its growth. At 50 hours the effects are not significant. The effects become more pronounced as the seed develops, with respect to both the length of the coleoptile and the magnitude of the potential. The reliability of potentials as a measure of radiation injury is discussed elsewhere (1).

It is interesting to note that while the range of variation of the potentials of wheat seeds was similar to that of corn seeds, with a 20-fold variation within a given group not being uncommon, the range of variation of...
the lengths of coleoptiles was much smaller. Neglecting those that failed to develop at all an eightfold variation within a given group was quite rare. This is to be expected since the length of the coleoptile is the result of the activity of the seed over a considerable period, so that individual variations are greatly reduced. On the other hand, the potentials indicate the activity of the seed at the time of measurement or at a time immediately preceding it, with consequent great variations.

This is consistent with the other observed fact; namely, that seedlings with large coleoptiles do not necessarily give large potentials, since the cell activity even of the most vigorous seed may be small during a given time interval. However, the average potential of a few seeds over a long period of time, or the equivalent, the average of potentials of a number of seeds at a given time is probably as good an indication of the vigor of the seed as the length of the coleoptile.

Bioelectric potentials of bean seeds

The beans chosen for this study were the Lady Bountiful, a hybrid, high-yielding type. The potential was measured from the point at which the seed was attached to the pod across to the opposite side.

Although the magnitude of the potential is about four times that of corn seeds at any period of their growth, the similarity of the growth curves of corn and bean seeds is very close. Because of the marked similarity, it was not considered necessary to include the bean-seed curves.

Determinations of the variations of the potential of bean seeds with weight were also carried out. Although the weight of the larger group was twice that of the smaller, no significant difference in potential was noted for the two groups.

Bioelectric potentials of Lima bean seeds

A few measurements of the bioelectric potential of Lima beans were made at different periods of their growth. The seeds were measured from tip to tip as this axis gave the largest potential. It was difficult to work with these seeds after 16 hours because they burst out of their outer shell and crack apart. This probably forms an injury current which obscures the true potential of the seed. Even before bursting the seeds are quite porous, and the physiological salt solution from the contact brushes quickly penetrates into the internal part of the seed and over the surface with a resultant shorting-out of the potential.

The average steady potential of the seeds just before bursting was about 3.5 mv with a range of 0.5–10 mv.

Summary

As a result of this study of the bioelectric potentials of certain seeds, the following conclusions can be made:

1. The high-yielding seeds have higher bioelectric potentials than those of low-yielding varieties.
2. The bioelectric potential of seeds is zero at dormancy and rises slowly during the first few hours in a humid environment. The potential rises much more rapidly as soon as coleoptile elongation begins.

3. The variation of bioelectric potential with x-ray dosage follows closely the variation of the length of coleoptile with dosage. X-rays, in general, decrease the bioelectric potential of plant seeds and lower their vitality.

4. The bioelectric potential is not a function of the weight of the seeds.

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