14. O. J. David, J. Clark, K. Voeller, *Lancet* **1972-II**, 900 (1972).
15. S. M. Pueschel, *Environ. Health Perspect.* 7, 13 (1974); A. M. Seppalainen and S. Hernberg, *Br. J. Ind. Med.* **29**, 443 (1972).
16. R. G. Lansdown, B. E. Clayton, P. J. Graham, J. Shepherd, H. T. Delves, W. C. Turner, *Lancet* **1974-III**, 538 (1974); P. J. Landrigan, R. H. Whitworth, R. W. Baloh, N. W. Staehling, W. F. Barthel, B. F. Rosenblum, paper presented at the Conference of Childhood Lead Poisoning Control Projects, Center for Disease Control, Atlanta, May 1974.
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Viability of Stored Seed: Extension by Cathodic Protection

Abstract. *Placing seeds on a negatively charged conductor extended their viability during artificial aging. Such cathodic protection may reduce free radical attack by providing a source of electrons. The results support the hypothesis of free radical damage to cellular components and are consistent with such damage being important in deteriorative senescence changes.*

It is well known that free radicals initiate peroxidative degradation of unsaturated tissue lipids, and it has been suggested that this could give rise to damage to cellular membranes (*1*). Lipid peroxidation in monomolecular and bimolecular films leads initially to an increase in membrane permeability and then to a decrease in membrane stability (*2*). Damage to biological membranes associated with lipid peroxidation has been shown in mitochondria (*3*), microsomes (*4*), and lysosomes (*5*). Lipofuscin granules accumulate with chronological age in some animal tissues and these granules appear to contain protein and peroxidized lipid (*6*), indicating that lipid peroxidation occurs throughout the life span of an organism. It has been suggested that this free radical peroxidation of unsaturated lipids is a basic deteriorative mechanism in cellular aging (*7*). There is as yet little direct evidence for the accumulation of membrane damage during senescence. However, extremely swollen mitochondria, with disorientated and fragmented cristae, indicative of membrane damage, have been observed in the flight muscle of old (25 to 30 days) male houseflies (*8*).

Attempts have been made to prolong the life span of organisms, and promising results have been obtained by supplying membrane-stabilizing drugs to *Drosophila* (*9*) and by including various antioxidants in the diet of mice (*7*, *10*). However, these results are far from unequivocal: not all treatments are effective (*7*, *9*), different results are obtained with the same antioxidant on different strains of mice (*7*), and in some experiments lipid peroxidation appeared to be reduced without any effect on life span (*11*).

Molnár (*12*) attempted to reduce free radical attack, not by supplying antioxidants in the diet, but by maintaining mice in a cage with an applied negative potential in a manner analogous to the technique of cathodic protection of metals against corrosion. The average and maximum life spans of mice exposed to a negative charge were, respectively, 25 and 32 percent greater than those of mice exposed to a positive charge.

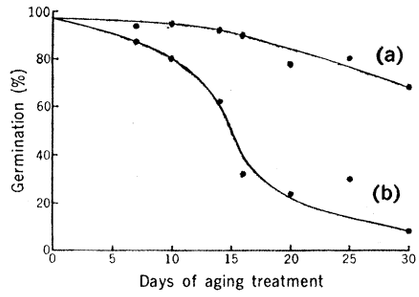

Fig. 1. Viability of stored seeds of *Zea mays* as a function of the duration of the aging treatment. Seeds with a moisture content of 13.6 percent were placed, embryo side down, on aluminum foil in a small enclosed chamber (to maintain the moisture content) and kept at 40°C to accelerate their aging (reduce their storage life). The entire contents of a single chamber were taken for each sample. The seeds were soaked in tap water overnight and then placed on moist paper toweling. Germination was recorded 84 hours after the start of soaking. (a) Seeds provided with cathodic protection by applying a negative potential of 300 volts to the aluminum foil; (b) control seeds.

Air-dry seeds can be rapidly aged artificially by maintaining them at a relatively high moisture content (13 to 15 percent) and an elevated temperature (40°C) (*13*). Air-dry seeds probably do not have sufficient moisture to permit aqueous-phase enzyme reactions to occur, and repair mechanisms are unlikely to be operative in such a system. Thus, damage induced by free radicals would accumulate in dry seeds, whereas in hydrated tissue such damage could be repaired (*14*). Seeds of *Zea mays* (Indian corn) that have been artificially aged show aberrations of mitochondrial membranes as an early sign of aging, before the viability of the seeds declines (*13*).

Seeds of *Zea mays* were subjected to artificial aging treatment by maintaining them in a small enclosed chamber at 40°C. The seeds were provided with cathodic protection by placing them, embryo side down, on aluminum foil within the chamber and applying a negative potential of 300 volts to the foil. Control seeds were maintained under similar conditions without the applied charge. The effect of storage time on the viability of the seeds is shown in Fig. 1.

Considerable decreases in viability loss were achieved with cathodic protection. In addition, the conductivity of the water in which the seeds were soaked before germination was consistently greater for the control seeds than for the seeds which had been exposed to the negative charge. This indicates that more material was leached from the control seeds during soaking, which suggests that greater membrane damage had occurred in them than in the seeds provided with cathodic protection. Chromosome aberrations are known to accumulate in nondividing cells of seeds under conditions of accelerated aging (*15*). Free radical peroxidation may affect macromolecules other than lipids; thus, our observation that after 10 days of the aging treatment the percentage of chromosome aberrations was 12.6 in the control seeds and 4.3 in the protected seeds may be of significance. The reductions in mortality rate for dry seeds, which are relatively nonhydrated systems, were far greater than any achieved for animals, which are hydrated systems.

Cathodic protection should reduce free radical attack on biological macromolecules by providing a source of electrons to react with the free radicals (*12*). Thus, these results provide strong evidence for free radical damage to

cellular components. In a system such as dry seeds, where repair mechanisms are probably inoperative, this damage could accumulate to such an extent that cell death and consequent loss of embryo viability occur. In hydrated systems, where membrane turnover (*1*), and thus presumably repair, does occur, membrane damage by free radical peroxidation probably does not accumulate (*14*). However, accumulation of this type of damage might well be important in the terminal deteriorative senescence changes in such systems if repair mechanisms fail with age.

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References

1. L. Packer, D. W. Deamer, R. L. Heath, *Adv. Gerontol. Res.* **2**, 77 (1967).
2. H. van Zutphen and D. G. Cornwell, *J. Membr. Biol.* **17**, 79 (1973).
3. F. E. Hunter, A. Scott, P. E. Hoffsten, J. M. Gerecki, J. Weinstein, A. Schneider, *J. Biol. Chem.* **239**, 614 (1964).
4. J. D. Robinson, *Arch. Biochem. Biophys.* **112**, 170 (1965).
5. I. D. Desai, P. L. Sawant, A. L. Tappel, *Biochim. Biophys. Acta* **86**, 277 (1964).
6. B. L. Strehler, *Adv. Gerontol. Res.* **1**, 343 (1964).
7. D. Harman, *Radiat. Res.* **16**, 753 (1962).
8. R. S. Sohal and V. F. Allison, *Exp. Gerontol.* **6**, 167 (1971).
9. R. Hochschild, *ibid.*, p. 133.
10. D. Harman, *J. Gerontol.* **23**, 476 (1968).
11. A. Tappel, B. Fletcher, D. Deamer, *ibid.* **28**, 415 (1973).
12. K. Molnár, *Mech. Age. Dev.* **1**, 319 (1972/73).
13. P. Berpak and T. A. Villiers, *New Phytol.* **71**, 135 (1972).
14. T. A. Villiers, in *Seed Ecology*, W. Heydecker, Ed. (Butterworth, London, 1973), p. 265.
15. F. H. Abdalla and E. H. Roberts, *Ann. Bot. (Lond.)* **32**, 119 (1968).

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Serological Detection of Mixed Lymphocyte Culture Identity between Cells That Differ by One HL-A Haplotype

Abstract. *Antibody mediated cell dependent immune lympholysis (ABCIL), an extremely sensitive serological technique for detecting tissue sensitization, was used in a family segregation study. Two serums capable of demonstrating ABCIL were used to identify members of the family who differed by one HL-A haplotype but whose cells did not stimulate in mixed lymphocyte culture. Absorption study of one serum indicated that the ABCIL reaction of that serum was directed against a factor, independent of HL-A, that might be responsible for lymphocyte stimulation in mixed lymphocyte culture. Thus the ABCIL technique may be used to detect lymphocyte-defined gene products.*

Antibody mediated cell dependent immune lympholysis (ABCIL) is a sensitive technique for detecting evidence of tissue sensitization in humans. We have shown previously that the antibody in the ABCIL reaction is different from that reacting in the complement dependent cytotoxicity test (*1*), that it is frequently positive in transfused patients who are nonresponders by the cytotoxicity test (*2*), and that the ABCIL specificity is not defined by HL-A (*3*). We now have evidence from studies of one large family that ABCIL reactivity segregates with the HL-A antigens. Our data also show that ABCIL reactivity may be used to detect mixed lymphocyte culture (MLC) factors. These MLC factors or lymphocyte-defined (LD) determinants have been postulated to exert as much or perhaps more control than HL-A over the outcome of tissue grafting between unrelated individuals (*4*).

The cells of the grandparents of the family were first screened for ABCIL reactions with the use of several serums whose specificity in the microlymphocytotoxicity test was known. Of the 22 serums so screened, two gave positive reactions with cells of only one grandparent, a different grandparent for each serum. As judged by the complement dependent lymphocytotoxicity test both serums have antibody against HL-A12. However, there is no HL-A12 in this family, and these ABCIL reactions cannot be explained in terms of their known HL-A reactivity.

With serum 1, the cells of the grandfather were negative, but the cells of the grandmother were positive, whereas the converse is true with serum 2 (Fig. 1). These two serums were then used with the cells of some of their children, their grandchildren, and their daughter-in-law Ruth. As is shown in Fig. 1, the positive ABCIL reactions of serum 1 can be shown to segregate with haplotype *c* (and haplotype *x*) if we assign *a* and *b* as haplotypes 2, W15 and 2, 5 of the father; and *c* and *d* as haplotypes 1, W17 and 2, W5 of the mother; and *x* and *y* as haplotypes 3, W5 and W32, W14 of the daughter-in-law. Similarly, positive ABCIL reactions of serum 2 segregate with haplotype *a*, although serum 2 is also weakly positive with the cells of grandchild Bruce, who carries haplotype *d* rather than the expected haplotype *a*. Apart from this weak reaction with serum 2, which we cannot explain, all other positive reactions are strong (*5*). Thus, in this family, these two serums contain ABCIL reactivities which segregate with two distinct HL-A haplotypes, *a* and *c*, one from each grandparent, and yet the complement dependent cytotoxic specificities are unrelated to the HL-A antigens of these haplotypes. These two haplotypes are both inherited by Eleanor and Don (Fig. 1).

By chance, each of these two ABCIL reactive serums also differentially mark Terry and Ruth, two parents in the second generation, although serum 1 is now marking haplotype *x* from outside the family (not haplotype *c*), and haplotypes *a* and *x* are inherited by Brian and Laurie in the third generation. That haplotypes *c* and *x* share characteristics is further established by absorption studies. These showed that ABCIL reactivity of serum 1 against haplotype *c* (Eleanor) could be removed by lymphocytes bearing haplotype *x* (Laurie), and vice versa. Terry's cells were used as controls in these absorption studies and did not remove ABCIL activity from serum 1. Results with platelet absorption were less conclusive, but suggested that ABCIL activity could not be absorbed by platelets at the dosage used (*6*). On the basis of these lymphoid cell absorptions, we believe that a factor linked to haplotype *c* may be identical to the factor linked to haplotype *x*, and that both are expressed on the lymphocyte membrane.

Hence, in this family, two HL-A identical sib pairs, Eleanor and Don in the second generation and Brian and Laurie in the third generation, have cells that behave identically by the ABCIL test with these two selected serums, both directly and by absorption. These two pairs of HL-A identical siblings have one HL-A haplotype in common: HL-A2 and W15, derived from the grandfather, and marked by serum 2. The other HL-A haplotypes, however, are different and are derived from unrelated sources; nevertheless both are marked by serum 1.