cent of the DNA would have been detected, this experiment indicates that, in the DNA-RNA hybrid, it is specifically the RNA moiety which is degraded by the enzyme present in fraction A.

Several of the degradation experiments described have been repeated with DNA-RNA hybrids synthesized with RNA polymerase from *E. coli* instead of from calf thymus. These hybrids showed essentially the same sensitivity to the action of fraction A. The enzyme has been purified some 50-fold by column chromatography and $(NH_4)_2SO_4$ precipitation. Of this preparation, 0.25 μg of protein solubilizes 100 pmole of RNA nucleotide from a DNA-RNA hybrid within 10 minutes at 37°C.

A variable degree of contamination of RNA polymerase preparations with the enzyme that splits DNA-RNA hybrids may explain the conflicting results described by others regarding the template activity of denatured DNA for mammalian RNA polymerase. Thus, depending on the purity of the polymerase preparations used, denatured DNA was not active as template at all (*3*), or it exhibited activity similar to that of native DNA (*2*). In contrast to that, our preparations show a fivefold higher template activity of denatured DNA (see Table 1).

The biological function of the hybrid-degrading enzyme is obscure. A function of DNA-RNA hybrids in the regulation of RNA synthesis has been suggested by Bekhor *et al.* (*9*). Such a mechanism might require an enzyme with the characteristics of the one described here.

Note added in proof: Further recent experiments have shown that the enzyme described above degrades polyuridylic acid if it is hybridized to polydeoxyadenylic acid but not if it is hybridized to polyadenylic acid. This further demonstrates the specificity of the enzyme.

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Salt Toleration by Plants: Enhancement with Calcium

Abstract. *Bean plants subjected to a sodium chloride concentration about one-tenth that of seawater for 1 week suffered no damage if the calcium concentration of the nutrient solution was 1 millimole per liter or higher, but at lower calcium concentrations damage was severe and apparently due to a massive breakthrough of sodium into the leaves.*

Of all the inhibitory substances that plants may encounter in their natural chemical environment, none impairs or prevents their growth on so large a scale as salt (*1*). About a quarter of the irrigated land in our own West is said to be affected by salinity (*2*). Saline conditions prevail in areas otherwise particularly well suited to crop production if water for irrigation is available; the unleached soils of these regions are inherently fertile, growing seasons are long, and temperatures and light intensities are high. Vast areas in developing countries belong in this category.

Calcium ions play a crucial role in the regulation of the salt economy of plants and specifically in the selective transport or exclusion of Na and other mineral ions by plant cell membranes (*3*, *4*). Despite this evidence, there seems to be little recognition of the role of Ca in affecting the responses of plants to saline conditions. The only three comprehensive compendia on the subject of salinity (*5*) make no mention of it. Hyder and Greenway (*6*) were concerned solely with the effect of NaCl at low levels of nutrients.

Brittle wax bush bean plants, *Phaseolus vulgaris* L., were first grown in a tenfold dilution of the nutrient solution described by Johnson *et al.* (*7*). After 14 days, they were transferred to fresh Johnson solution (1/10) lacking $Ca(NO_3)_2$ but containing NaCl at 50 m*M* and graded additions of $CaSO_4$ as indicated in Fig. 1. Control plants were grown in cultures otherwise identical with these but containing no NaCl.

The plants grew in these solutions for 7 days. The experiment was then discontinued, and fresh and dry weights of the roots, stems, and leaves were taken. Both Na and K were determined by flame spectrophotometry, and Ca and Mg were determined by atomic absorption spectrophotometry.

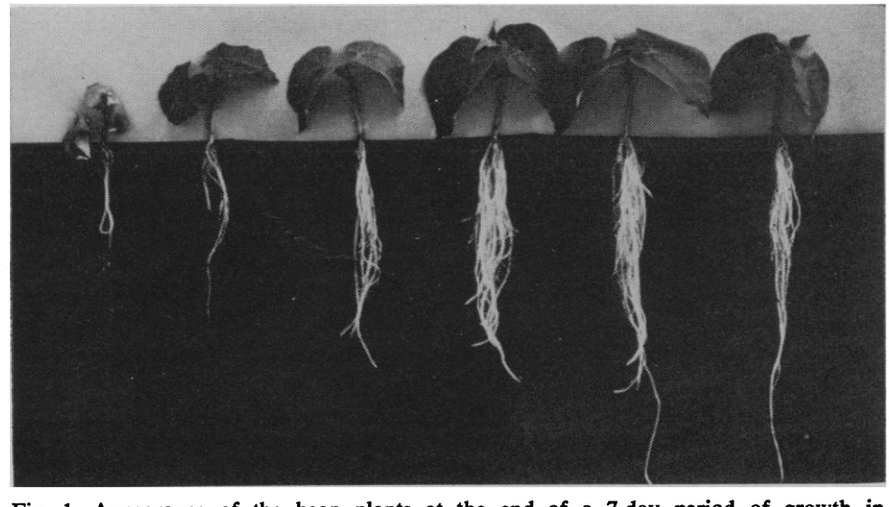

Fig. 1. Appearance of the bean plants at the end of a 7-day period of growth in aerated nutrient solutions in the greenhouse. The concentration of NaCl was 50 m*M*, and that of $CaSO_4$ was (left to right) zero, 0.1, 0.3, 1.0, 3.0, and 10 m*M*.

The plants exposed to 50 mM NaCl at $CaSO_4$ concentrations below 1 mM suffered great damage (Fig. 1). At higher concentrations of $CaSO_4$, the plants grew well and, both in appearance and weight, did not differ significantly from the control plants receiving no NaCl. All control plants grew equally well, except those receiving no Ca.

The Na concentration in the roots dropped by about one-third as the $CaSO_4$ concentration in the solutions was raised from 0.1 to 10 mM. Over the same interval of $CaSO_4$ concentrations, the Na content of the stems fell by a factor of about 2.5. However, it was in the leaves that the effect of $CaSO_4$ on Na content was most dramatic. The Na concentration fell from 3.2 mg per 100 mg (dry weight) at 0.1 mM $CaSO_4$ to 0.2 mg per 100 mg at 3 mM. There was no further change at 10 mM $CaSO_4$. That is, at less than 3 mM $CaSO_4$, a massive breakthrough of Na into the leaves occurred.

The effect of $CaSO_4$ is due to the Ca ion; repetition of the experiment with $CaCl_2$ instead of $CaSO_4$ gave very similar results. Attempts to replace Ca with either Mg or K (in the presence of 0.1 mM Ca) proved futile, affording little or no protection. At the effective concentrations of Ca, Mg caused damage in addition to that attributable to NaCl.

The protective action of Ca is not related to any simple shift in internal cationic ratios without change in the total cationic content. This is evident from the fact that the changes in Na content of the tissues, induced by changes in the Ca concentration of the solutions, far exceeded any changes in the concentrations of the other cations in the tissues.

Both cellular membranes and RNA may be sites of Na-induced derangements and, specifically, displacement of Ca, as already surmised on the basis of other evidence (*4*, *8*). Since a massive intrusion of Na into the plant was the outstanding effect of salinity, both this intrusion and the decline of the plants being checked by Ca, it is likely that the site of the primary Na (and Ca) effect is the plasmalemma of the absorbing cells of the roots, for that is where entry of ions into the plant is governed (*9*).

Our experiments show the efficacy of Ca in protecting an extremely salt-sensitive species against the deleterious effects of NaCl present in the medium at about one-tenth its concentration in seawater.

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Rat Heart Papillary Muscles: Action Potentials and Mechanical Response to Paired Stimuli

Abstract. *Electrical reexcitation of rat papillary muscle after a short interval (50 to 80 milliseconds) results in action potentials with no significant mechanical counterpart. The mechanical response recovers as the interval is increased beyond 80 milliseconds. The rate of recovery is slowed at low external calcium levels. It appears that the coupling mechanism passes through a refractory stage owing to the depletion of an intracellular "releasable calcium" fraction.*

Rat papillary muscle fibers have short action potentials compared with those found in ventricular muscle of other species (*1*). This provides an opportunity to investigate the effects of electrical reexcitation during the period of tension development, a technique that proved useful in characterizing the active state of skeletal muscle (*2*, *3*).

In order to study the effects of paired pulses, papillary muscles were isolated in a plexiglass bath and stimulated with either a pair of fine-wire platinum electrodes or platinum plate electrodes placed on either side of the muscle, 1 mm away. A modified Tyrode solution (*3*) was used to bathe the muscles. Most experiments were performed at 26°C, since this gave a better separation of electrical and mechanical activity than at higher temperatures. Intracellular action potentials were measured with glass microelectrodes, and tension was recorded on an oscilloscope by using a Statham G 1-4 strain gauge.

When paired stimulus pulses were used to excite the preparation, two action potentials could be recorded. The amplitude of the second action potential became slightly smaller than that of the first as the interval between the stimuli decreased to less than 100 msec, but the decrease was small (less than 10 percent) for most fibers until the interval was reduced to 50 msec. At shorter intervals the second action potential could not always be elicited though in some preparations double action potentials could be seen at stimulus intervals as short as 30 msec. The mechanical response on the other hand became progressively smaller as the interval decreased until at pulse intervals of 80 to 100 msec the effect of the second pulse became practically undetectable (Fig. 1). Neither the peak tension nor the duration of the concentration due to a single pulse could be increased significantly by a second pulse falling at an interval of less than 80 msec after the first. Peak tension is increased when the second pulse falls later than 80 msec but before the peak which, at this temperature, falls 200 msec after the first impulse.

The value Tr, which is the relative increase in area under the tension time curve due to the second pulse, expressed as a fraction of the area resulting from a single pulse, was calculated from planimeter measurements. We found Tr to be indistinguishable from zero ($< .02$) at pulse intervals of 80 msec or less. At longer pulse intervals Tr increases steadily to a value of 0.3 at 200 msec. Recovery is more rapid at higher temperatures, and at 35°C Tr becomes measurable ($> .02$) at 50 msec and increases to 0.5 at 200 msec; but the response to the second pulse is much less than the first, even when the second pulse falls after the mechanical