EFFECTS OF n-DECENYLSUCCINIC ACID (n-DSA) AND ITS MONOMETHYL ESTER (m-MDSA) ON WATER AND ION FLUX IN ISOLATED ROOTS

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CHAPTER I

INTRODUCTION

It has been known for some time that certain hydrocarbon compounds bring about a profound change in plant membrane permeability (1). Van Overbeek and Blondeau (10) stated that phytotoxic hydrocarbons acted by inducing separation of the lipid layers in the membrane, thereby increasing the permeability of the membrane(s).

Kuiper (4) reported in 1964 that exposing bean roots to a 10^{-3} M solution of n-decenylsuccinic acid (n-DSA) resulted in a gradual increase in water uptake amounting to nearly 800 per cent of the original value after two hours. He attributed the effect to the incorporation of the acid into the lipid layers of the cytoplasmic membranes and thus changed the membrane from a phase, characterized by a high potential energy barrier for water transport, to a phase in which only the effect of the viscosity of water is observable.

Kuiper, in an experiment with an analogous saturated compound, decylsuccinic acid, obtained a reduction in water uptake of 55 per cent. He reasoned that the double bond in the hydrocarbon chain was essential for the increase in water permeability. In another experiment, he found the temperature dependence of water uptake of bean roots was almost eliminated by treating with n-DSA. His data showed a drop in Q_{10} value from 3.8 to

1.2 two hours after application of a 5×10^{-4} M n-DSA to the environment of the roots.

n-DSA and some other alkenylsuccinic acids were further reported to induce resistance to freezing and desiccation in strawberry flowers, bean seedlings, etc. (5, 7), to decrease temperature dependence of photosynthesis in bean plants (6) and to reduce transpiration of leaves through stomatal closure in tobacco leaves (11).

Kramer et al more recently (9) confirmed the increase in permeability of bean root systems to water by n-DSA at 10^{-3} M, but found that 10^{-4} M n-DSA reduced the permeability. They also found that both concentrations caused leakage of salts from the roots and cessation of root pressure exudation. They proposed that n-DSA may be acting as a metabolic inhibitor at low concentrations and that the increase in water permeability to higher concentrations of n-DSA was the result of injury to the roots.

Most of the foregoing workers measured the water flux in isolated roots using a potometric method that involved the application of suction to the cut ends which tended to bias the observed exudation of both water and salts. Moreover, in the salts studies, only changes in the conductivity of the exudate were measured, which gave no information as to what specific ion(s) was involved in the n-DSA effect.

The purpose of this study, then, was (1) to measure the changes in water flux in isolated roots exposed to n-DSA and its mono-methyl ester (m-MDSA) using a potometric technique in the absence of an externally applied suction and (2) to measure the flux of Na and K in similarly treated roots using a flame photometric method.

CHAPTER II

MATERIALS AND METHODS

Onion bulbs (Allium cepa, var., White Ebenezer) were sprouted in continuously aerated full strength Hoagland's solution. The solution was renewed monthly. Bulbs weighing between four and eight grams were supported on perforated plexiglass strips over an aquarium containing twenty liters of the nutrient solution. The outer dry skin of the bulbs were removed and their bases brushed and cleaned. The bulbs were slightly immersed. The roots were grown in the dark to insure maximun root growth. Temperature during growth was 23 $\pm$ 2° C with the relative humidity ranging between 55 per cent and 60 per cent. At the end of the growth period (4 - 5 days), healthy, straight roots ranging from 55 to 100 mm in length were selected for the experiments.

The test compounds, n-decenylsuccinic acid and its mono-methyl ester were purchased from Humphrey Chemical Inc., North Haven, Conn. n-DSA has a formula of CH_3-$(CH_2)_6$-CH=CH-CH_2-CH(COOH)-CH_2-COOH and is a waxy, amorphous solid. Kuiper (8) used a few drops of hot ethanol to help bring it into solution; however, I had no problem in dissolving it in distilled water at a concentration of 10^{-3} M. After dissolving, its pH was about 4.1. In order to enhance its penetration into the roots, a few drops of 0.1 N KOH were added to raise its pH to 6.0 (3). The mono-methyl ester of n-DSA has a formula of CH_3-$(CH_2)_6$-CH=CH-CH_2-CH(COOH)-CH_2COOCH_3 and is a yellowish viscous liquid. It is insoluble in water but is soluble in

ethyl or isopropyl alcohol. For this reason, the test solutions of m-MDSA were made up in 1 per cent ethanol. The pH of a 1 x 10^{-3} M m-MDSA solution ranged between 4.2 and 4.5. Again, to enhance absorption by the roots, the pH of the test solution was fixed at 6.0 with 0.1 N KOH.

Prior to each experiment, the roots were excised in a Petri dish containing aerated Hoagland's solution and allowed to stand at least one hour. This was done to permit each root to recover from excision and to reduce possible water deficits. Following the equilibration period, an individual root was placed in a micropotometer similar to the one described by Lott and Wall (8). In most experiments, it took approximately two to three minutes to load the potometer. The process of loading the potometer was carried out as rapidly as possible to prevent dehydration. The total lehgth of the root immersed in the well in each experiment was 50 millimeters. The solution used in the well during the control and recovery periods was either aerated distilled water or aerated 1 per cent ethanol. Only fresh test solutions were used in the experiments.

Immediately following loading, the potometric apparatus was placed in a closed plexiglass chamber. Moist filter paper strips, partially immersed in the water bath chamber were placed on the sides to insure high humidity inside the chamber throughout the experiment. After positioning in the chamber, the root was allowed to equilibrate for about 15 minutes before the initial reading was taken. The construction of the chamber was such that solutions in the well surrounding the root could be changed without removing the potometer from the moist chamber. The ports on the sides of the chamber also allowed removal of samples from the potometer and well solution without disturbing the roots.

The volume of water efflux was measured at 15 minutes intervals by means of a horizontal microscope equipped with a micrometer eyepiece. Since the diameter of the potometer could be determined, a volume constant was derived. Thus, the volume of water efflux pumped for any unit of time could be determined by multiplying the constant by the distance the meniscus moved in the potometer.

The experiments in this study were divided into two parts. Part I involved water efflux measurements while Part II was concerned with the flux of Na and K. All of the test experiments consisted of a control period ranging between 30 minutes and an hour, a test period ranging between one hour and three hours, followed by a recovery period ranging between one and three hours.

Water Efflux

This study was divided into a control series and a test series of experiments. In the control series, eight runs were made to determine the rate of water efflux when the roots were exposed to aerated water for a period of five hours. In another set of control experiments, eight runs were made in which the effects of 1 per cent ethanol solution (pH 6.0) on water efflux were measured.

In one set of test experiments the roots were exposed to 1×10^{-3} M n-DSA in water (pH 6.0) for one hour while in another series the roots were exposed to 1×10^{-3} M n-DSA in water (pH 6.0) for a period of 2 hours and 45 minutes.

A third series of test experiments involved exposing the roots to 1×10^{-3} M m-MDSA (pH 6.0) for a period of two hours.

Ion Flux

This study was divided into a control series involving measurements of the changes in Na and K content in the effluent, the well solutions, and ashed roots. A test series involved the determination of changes in Na and K flux in roots exposed to either n-DSA (1×10^{-3} M) in water or m-MDSA (1×10^{-3} M) in 1 per cent ethanol.

In the first two phases of the cation flux study, five-microliter samples of the effluent and well solutions were withdrawn at the end of each of the three periods (control, test, and recovery), into a Hamilton #7005 N microsyringe. Following each sample removal from the potometer, the remaining fluid in the potometer was removed and three microliters of distilled water was added to establish the meniscus for the next sampling. The samples were then diluted to two milliliters volume, with 15 meq Li/liter lithium diluent, and analyzed for K and Na on a Model 143 Flame Photometer manufactured by Instrumentation Laboratory, Inc., Boston, Mass. The Na and K concentrations were determined from the same sample with a digital readout on the photometer by presetting the machine with flame photometer standards prepared by the same company.

At the end of the recovery period of each experiment, the root was removed and carefully washed in deionized water. The roots were then placed in acid-cleaned crucibles, dried in an oven at 130° C for one hour, and weighed. The crucibles were then placed in a burner and the contents ashed. The ash was dissolved in two millimeters of 15 meq Li/liter lithium diluent and analyzed for K and Na content as above. In one control series of experiments involving ten roots, the roots were taken from the growth chamber, soaked in water for one hour in a Petri dish, and then analyzed for K and Na content, using the foregoing methods.

CHAPTER III

RESULTS

Water efflux data are presented in terms of microliters efflux per millimeter of root length per reading (μl/mm root/15 min.). The ion efflux data are presented either as milli-equivalents per liter of sample, or in the case of the ashed roots, the cation contents were presented as milli-equivalents per 1 mg of dried root sample (meq/mg root). Each circle in Figures 1 through 6 represent mean values for at least eight roots.

Water efflux : Figure 1 shows the water efflux from isolated onion roots in distilled water over a period of five hours. It may be seen that at one hour following loading, a relatively steady rate of efflux was attained. Figure 2 shows the effects of exposing the roots for a period of three hours to 1×10^{-3} M n-DSA solutions (in water at pH 6.0). Readings were taken immediately upon loading the root in the chamber, and following a one-hour soaking period in aerated Hoagland's solution. During the first hour in aerated distilled water, a decrease in water efflux in the roots was noted. After one hour in n-DSA, a noticeable and sustained increase in the rate of water efflux occurred over a period of two hours. During the recovery period, the rates of water efflux returned to control levels within an hour, indicating reversal in the n-DSA effects. Figure 3 shows the effects of exposing roots to n-DSA (1×10^{-3} M, pH 6.0) for a period of one hour , then returning them to distilled water. From the curve one can observe an apparent "dilution" effect of n-DSA on the water

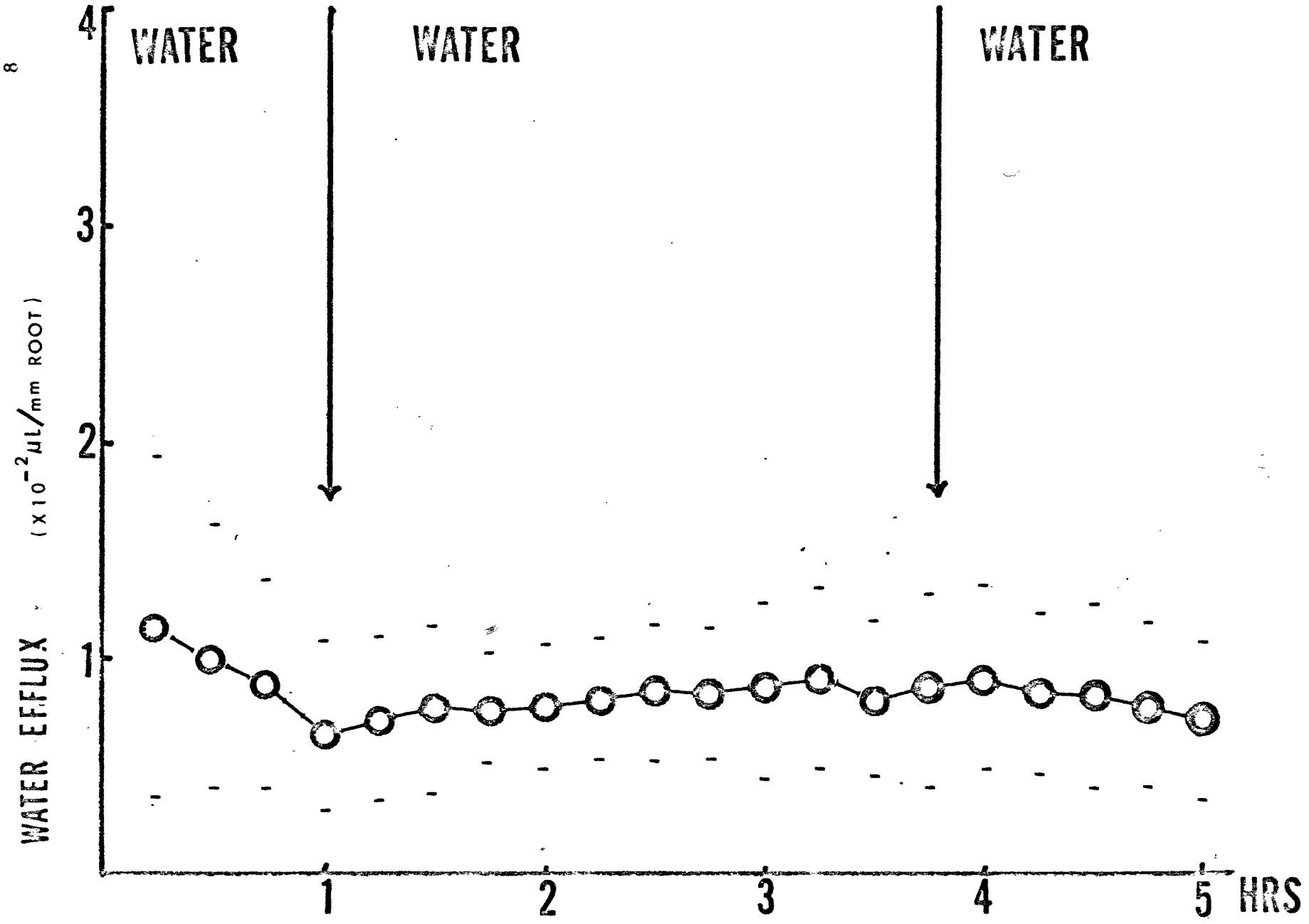

FIG.1 WATER EFFLUX IN ISOLATED ONION ROOTS

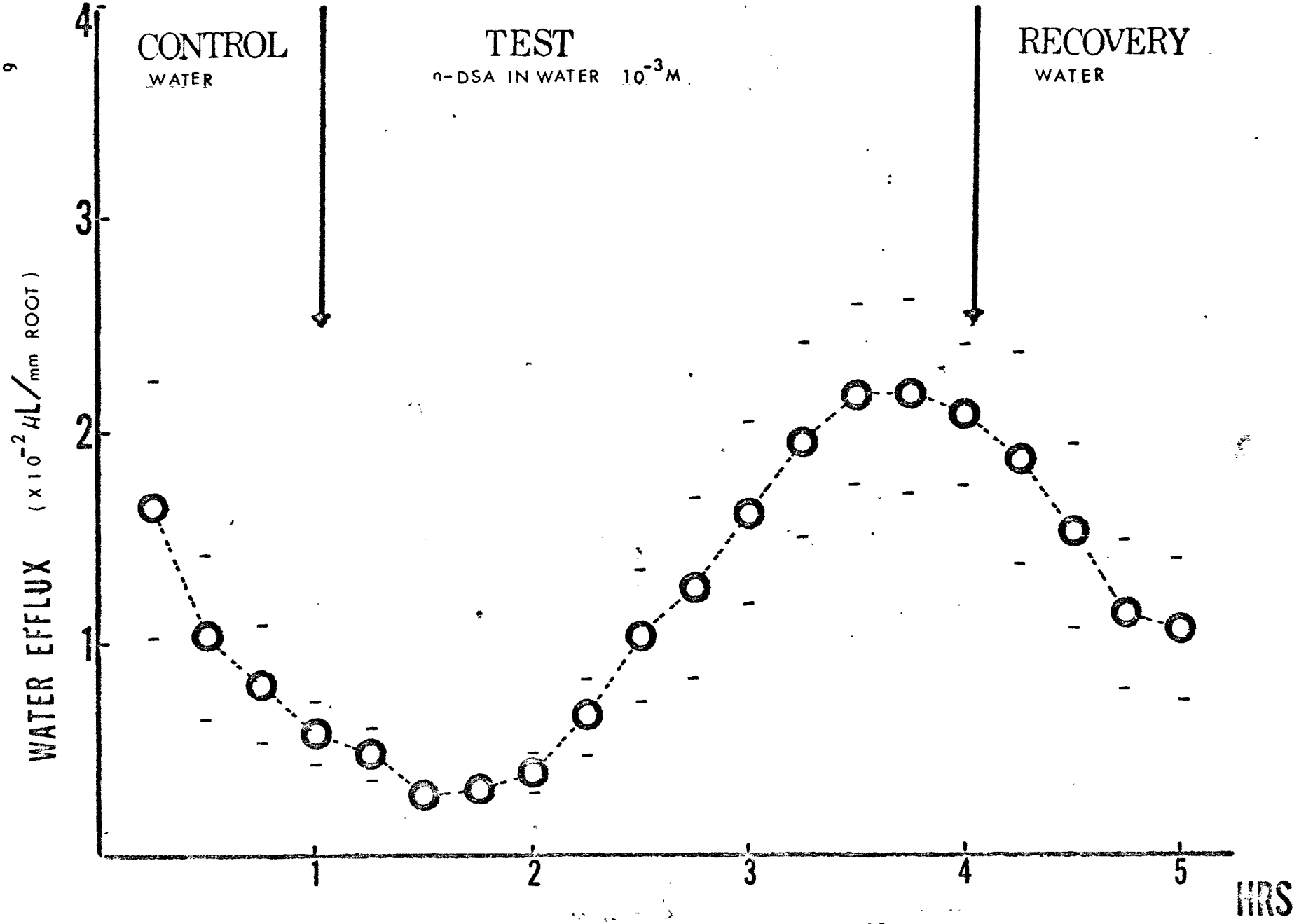

FIG.2 EFFECT OF N-DSA ON WATER EFFLUX

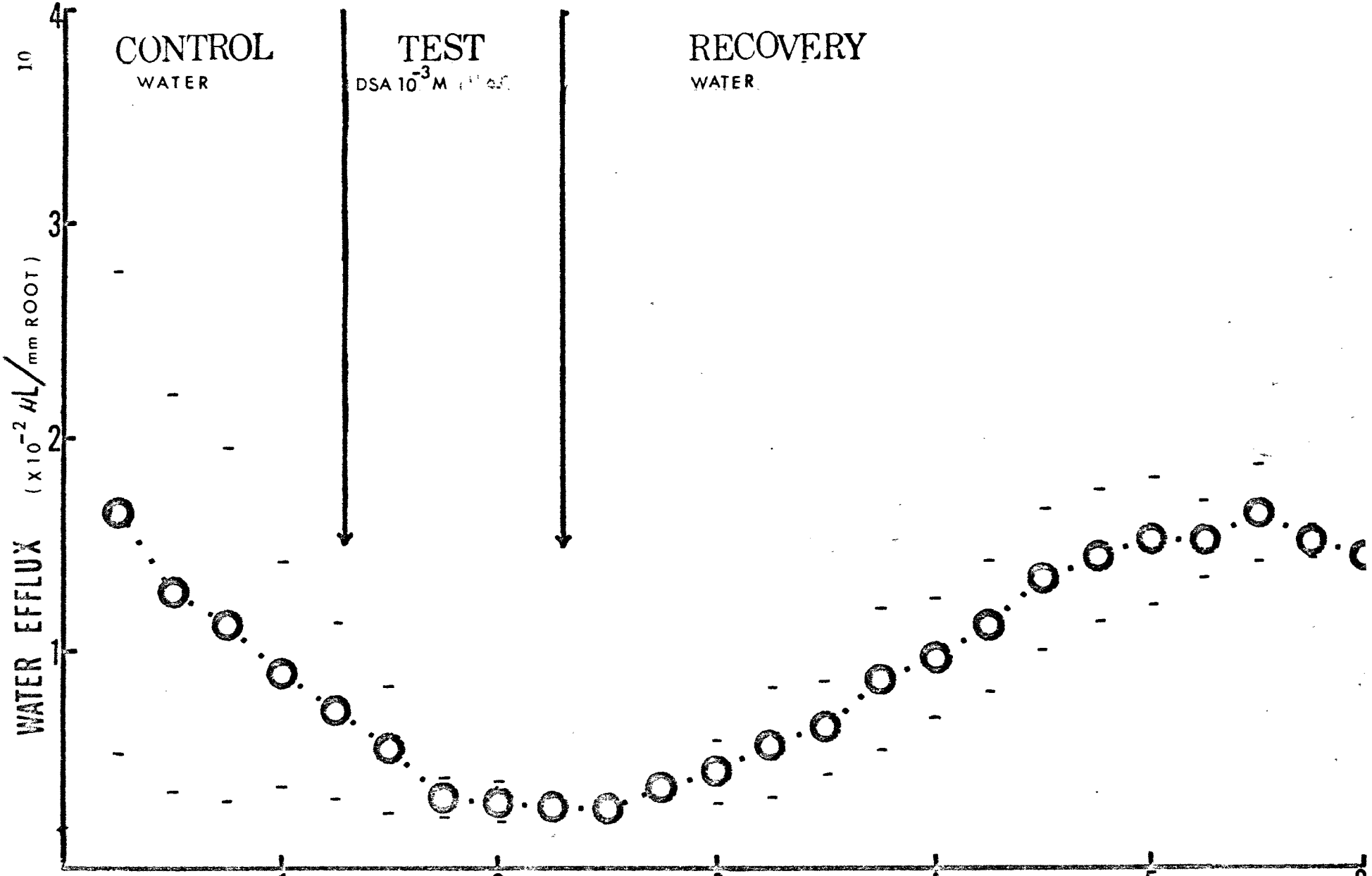

FIG.3 EFFECT OF N-DSA ON WATER EFFLUX

efflux. In the two hours following the return of the n-DSA treated roots to distilled water, a noticeable and sustained increase in the water efflux of the roots occurred. Figure 4 shows the effects of exposing isolated roots to 1×10^{-3} M m-MDSA (in water at pH 6.0) for a period of two hours. The stimulatory effect of this substance was striking in that it occurred within 30 minutes and lasted about one hour in all roots tested. No recovery was noted in any root. Indeed, the water efflux observed in the recovery period was almost nil after one hour, indicating an irreversible effect of the m-MDSA. Since m-MDSA proved to be more soluble in ethanol, another series of runs were made to test the effects of a 1 per cent solution of ethanol on water efflux (Figure 5). Relatively little effect of 1 per cent ethanol was observed in any of the roots during the test period. However, when the treated roots were returned to aerated distilled water, a slight increase in water efflux occurred. This effect was sustained for a period of 2 hours.

Figure 6 shows the effects of exposing the roots to an ethanolic solution of 1×10^{-3} M m-MDSA (pH 6.0) on water efflux. The results were found to be dissimilar to those observed in the roots exposed to m-MDSA in water in two respects : first, the stimulatory effect occurred more quickly and second, the effect was sustained for a slightly longer period of time. Again, no recovery was observed in the roots.

Summarily, the general effect of both n-DSA and m-MDSA was stimulatory in nature in regard to water efflux. The basic differences between the effects of the two substances was in the time, amplitude of duration and reversibility. Unlike m-MDSA, the effects of n-DSA required a longer period of time to occur, was of longer duration, was less in amplitude and appeared to be reversible.

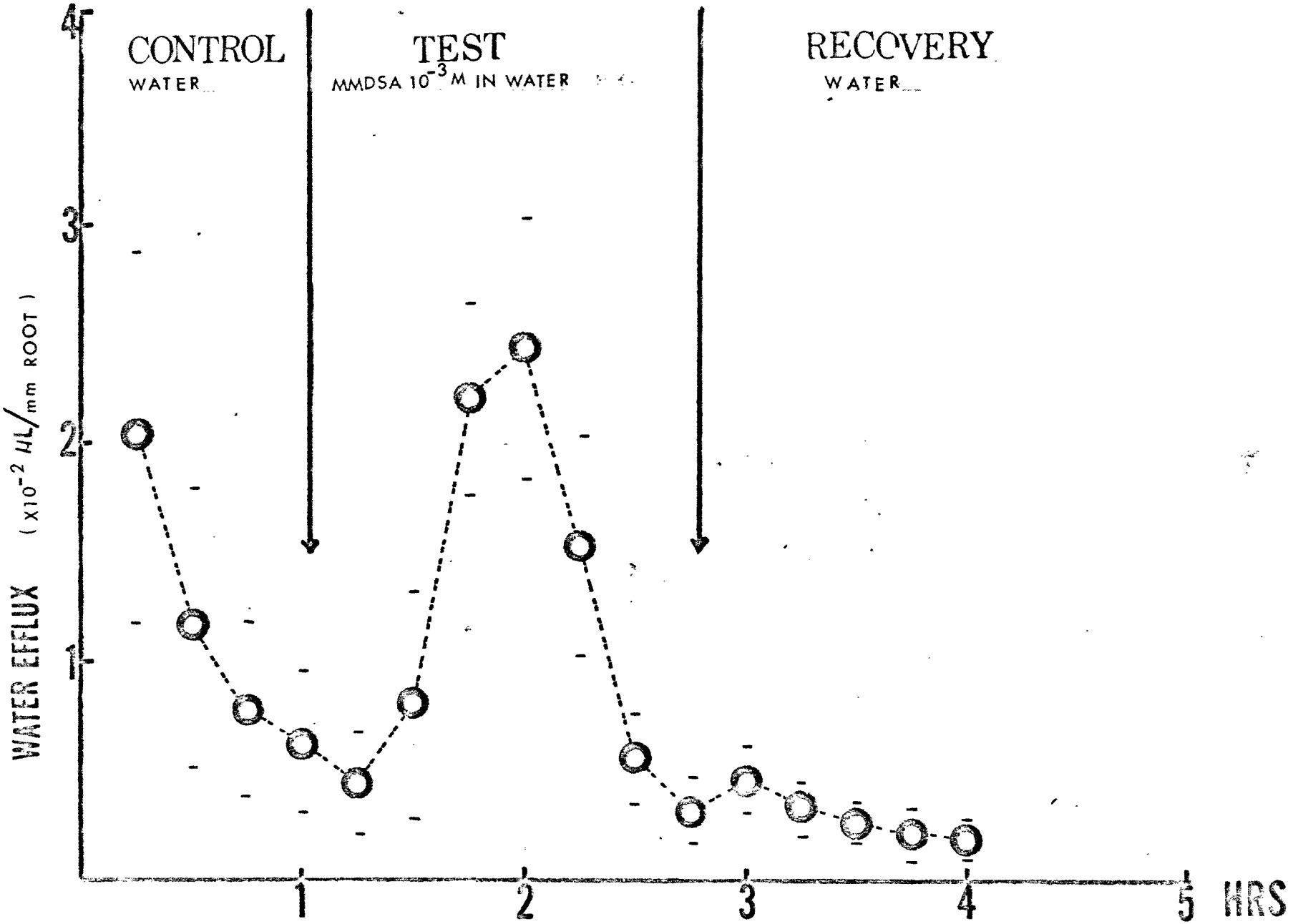

FIG.4 EFFECT OF MONO-METHYL ESTER OF N-DECENYLSUCCINIC ACID ON WATER EFFLUX

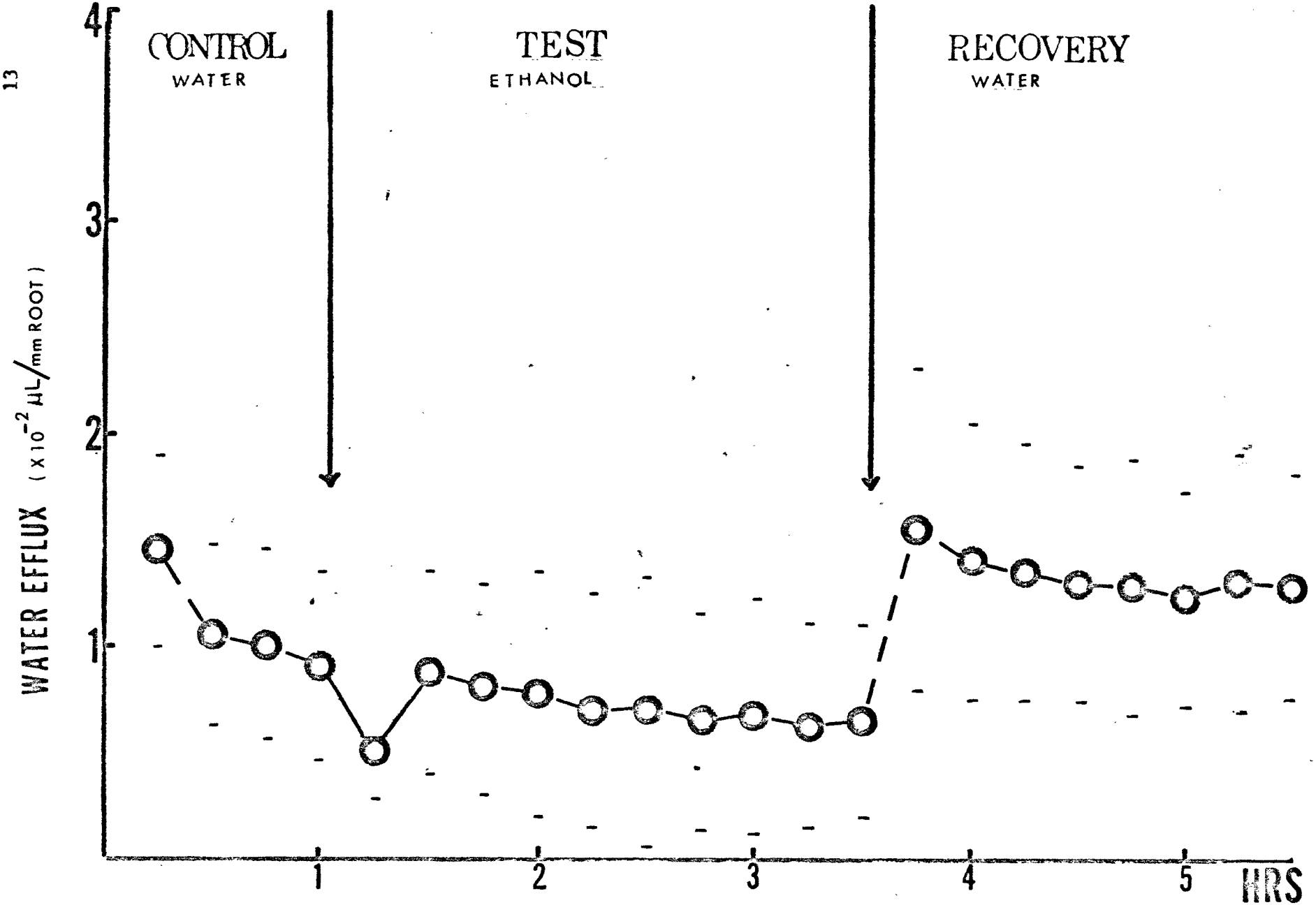

FIG. 5 EFFECT OF 1% ETHANOL ON WATER EFFLUX

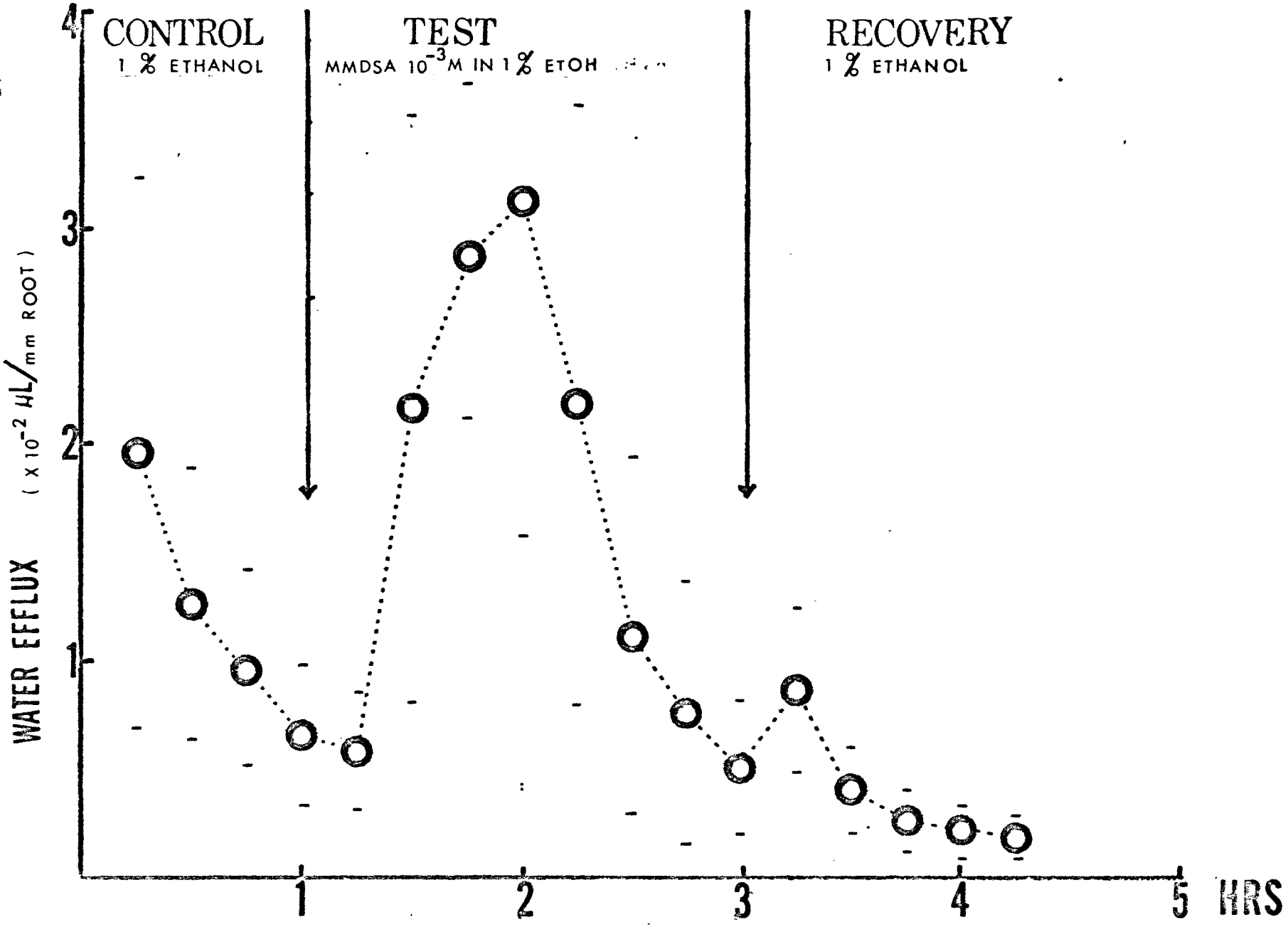

FIG.6 EFFECT OF MONO-METHYL ESTER OF N-DECENYLSUCCINIC ACID ON WATER EFFLUX

Potassium and sodium efflux : The data are depicted in Figures 7 through 10 as bar-graphs and summarized in Table 1. Again, each bar-graph depicts the mean values of eight roots. In this study, changes in K and Na concentrations in the effluent solution, the well solution, and in the ion content of the ashed roots were measured.

Figure 7 and Table 1 show the K and Na efflux in roots in aerated distilled water for a period of six hours. The data from the effluent samples clearly indicate a decrease in the loss of K from the basal end during the experiments, being significantly (at the 0.05 level) greater than the initial two hours. This finding was expected since the roots may still be showing the effects of cutting during this time. Since no significant change in K content of the well solution was observed in the same six-hour period, the K in the effluent solutions represented a loss from the root itself. On the other hand, no significant change in the Na content of either the effluent or the well solution was observed. Again, the larger Na content of the effluent solution during the first two hours indicated a loss of Na from the root itself.

Figure 8 and Table 1 show the effects of 1×10^{-3} M n-DSA on the K and Na efflux from roots. The data clearly show a significant (at the 0.05 level) increase in the K content of the effluent sample. This would indicate a loss in K from the root. The significant (at 0.05 level) increase in the K content of the well solution was thought to be due in part to the K added to the distilled water when fixing the n-DSA to pH 6.0 with 0.1 N KOH. During the recovery period, the K efflux returned to control levels indicating a reversible effect. No statistically significant change in the Na content of either the effluent or well solution was observed in roots exposed to n-DSA.

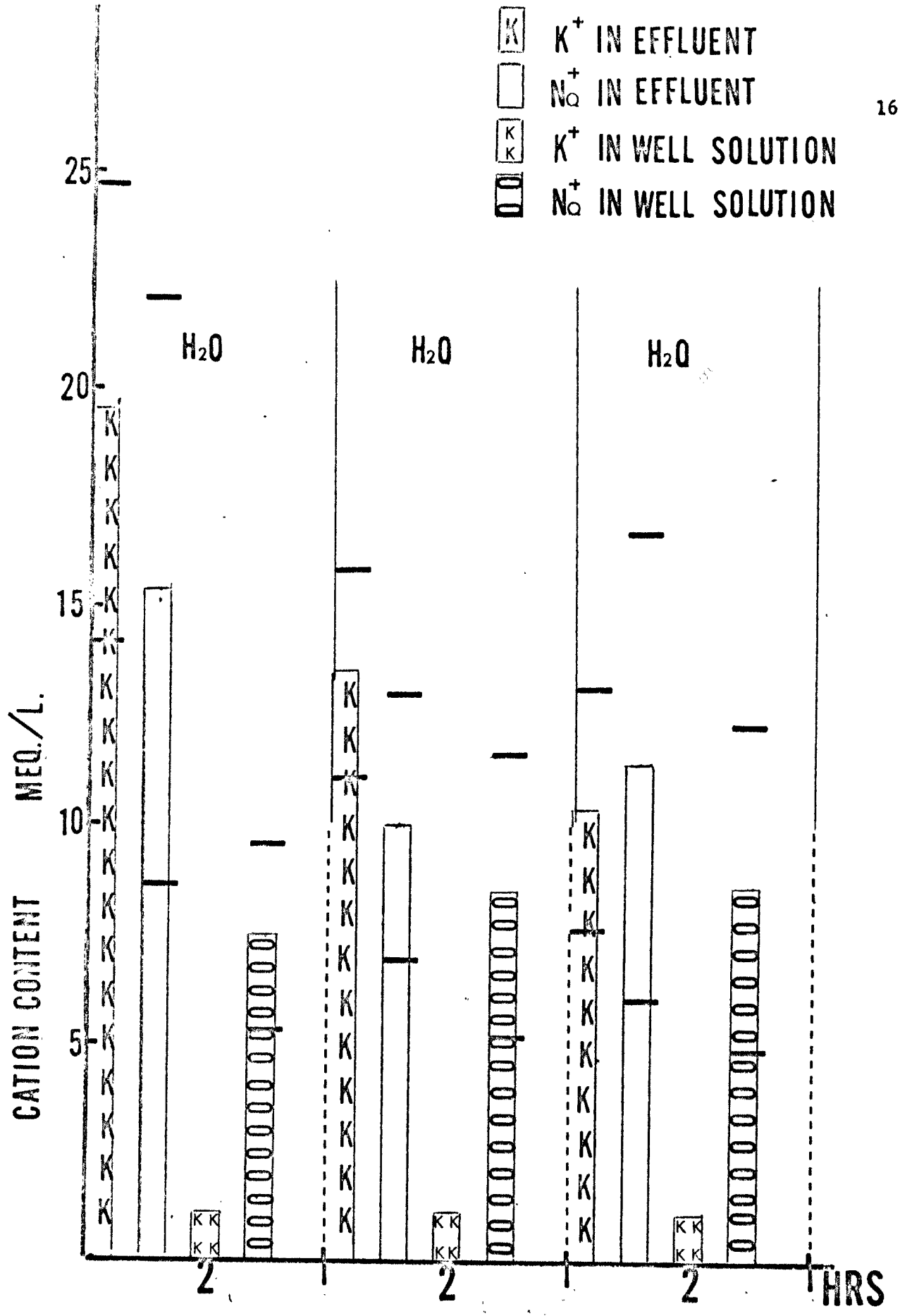

FIG.7 ION EFFLUX IN ISOLATED ROOTS

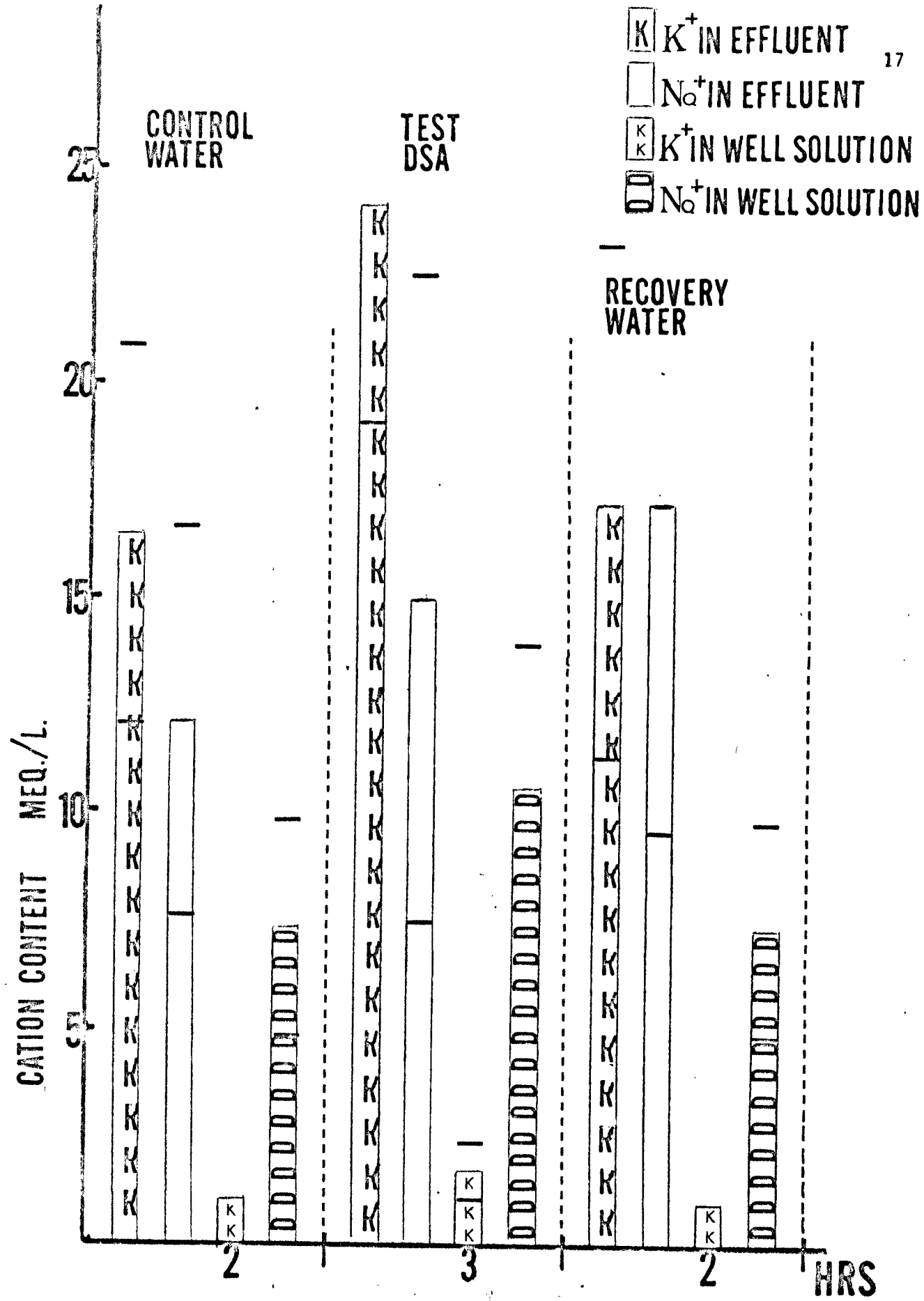

FIG.8 EFFECT OF N-DSA ON ION EFFLUX

Figure 9 and Table 1 show the effects of 1×10^{-3} M m-MDSA in ethanol (pH 6.0) on the K and Na efflux from isolated roots. Unexpectedly, no significant change in either the K or Na content of the effluent was observed in any roots tested. On the other hand, a statistically significant (at the 0.05 level) increase in the K content of the well solution was noted when the roots were exposed for two hours in 1×10^{-3} M m-MDSA. This would indicate a loss of K from the root surface rather than the cut basal end. This finding, however, may be partially attributed to the added K used in fixing the pH of both the 1 per cent ethanol and the m-MDSA solution.

Figure 10 and Table 1 contain data showing the changes in the Na and K content of ashed roots. Figure 10A shows data from experiments in which the roots were ashed one hour after placement in distilled water. Figure 10B indicates the efflux of Na and K from roots exposed to distilled water for 6 hours before ashing. Figure 10C shows data from runs in which the roots were exposed to distilled water for two hours, then to n-DSA for 3 hours, followed by exposure to 2 hours of distilled water before ashing. Finally, Figure 10D shows the effects of m-MDSA on Na and K efflux from roots exposed to distilled water 2 hours, to m-MDSA 2 hours, then returned to distilled water 2 hours before ashing. In the n-DSA and m-MDSA treated roots, one may, therefore, assume that some of the effects of both n-DSA and m-MDSA might have been masked or diminished by the 2 hours recovery period in distilled water prior to ashing. That the increase in K content of the well solution in roots treated with both n-DSA and m-MDSA may not be completely due to the added K in fixing the pH was indicated by the high percentage of decrease in the K content of the ashed roots (-82 per cent in n-DSA; -69 per cent in m-MDSA).

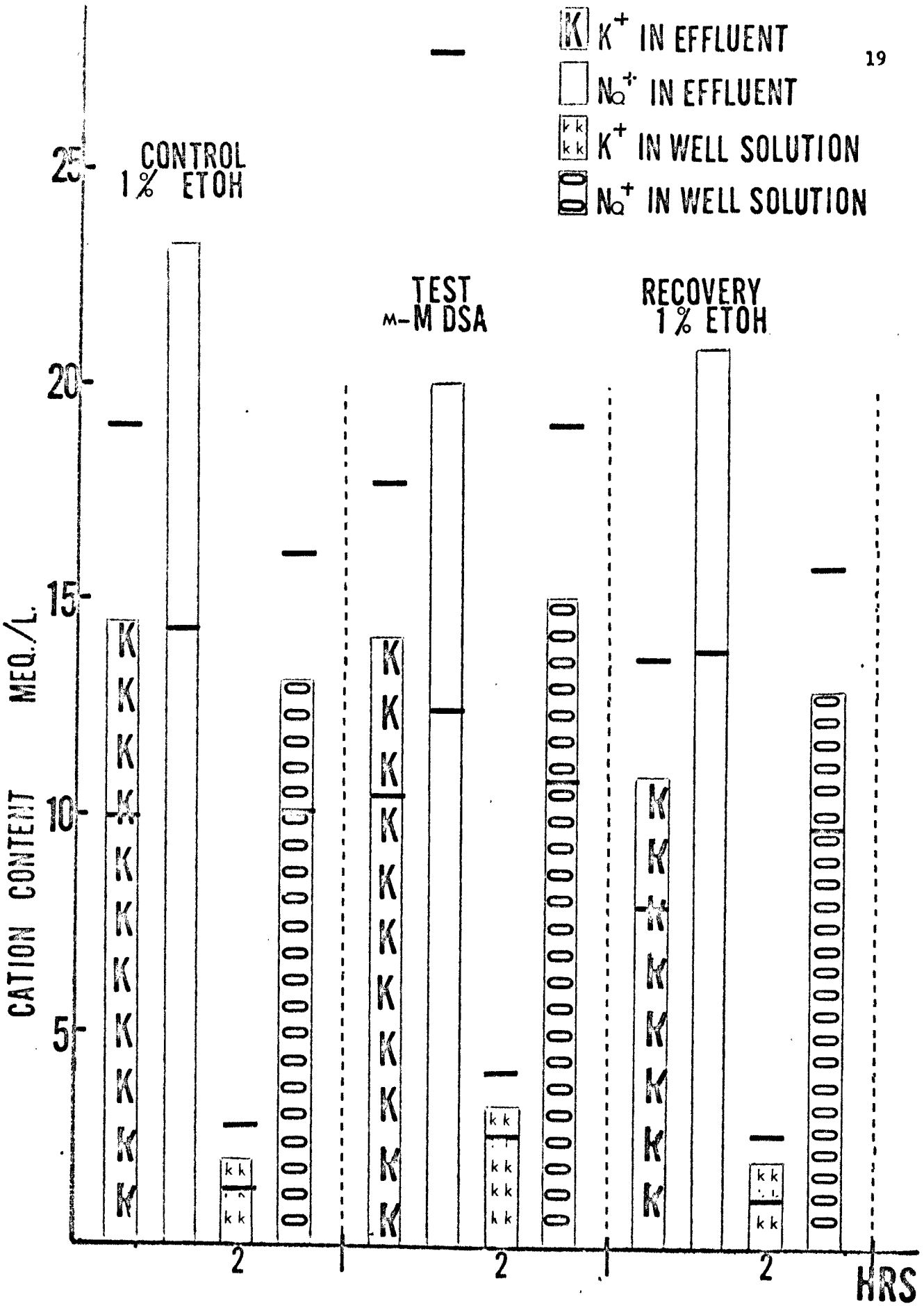

FIG.9 EFFECT OF MONO-METHYL ESTER OF N-DECENYLSUCCINIC ACID ON ION EFFLUX

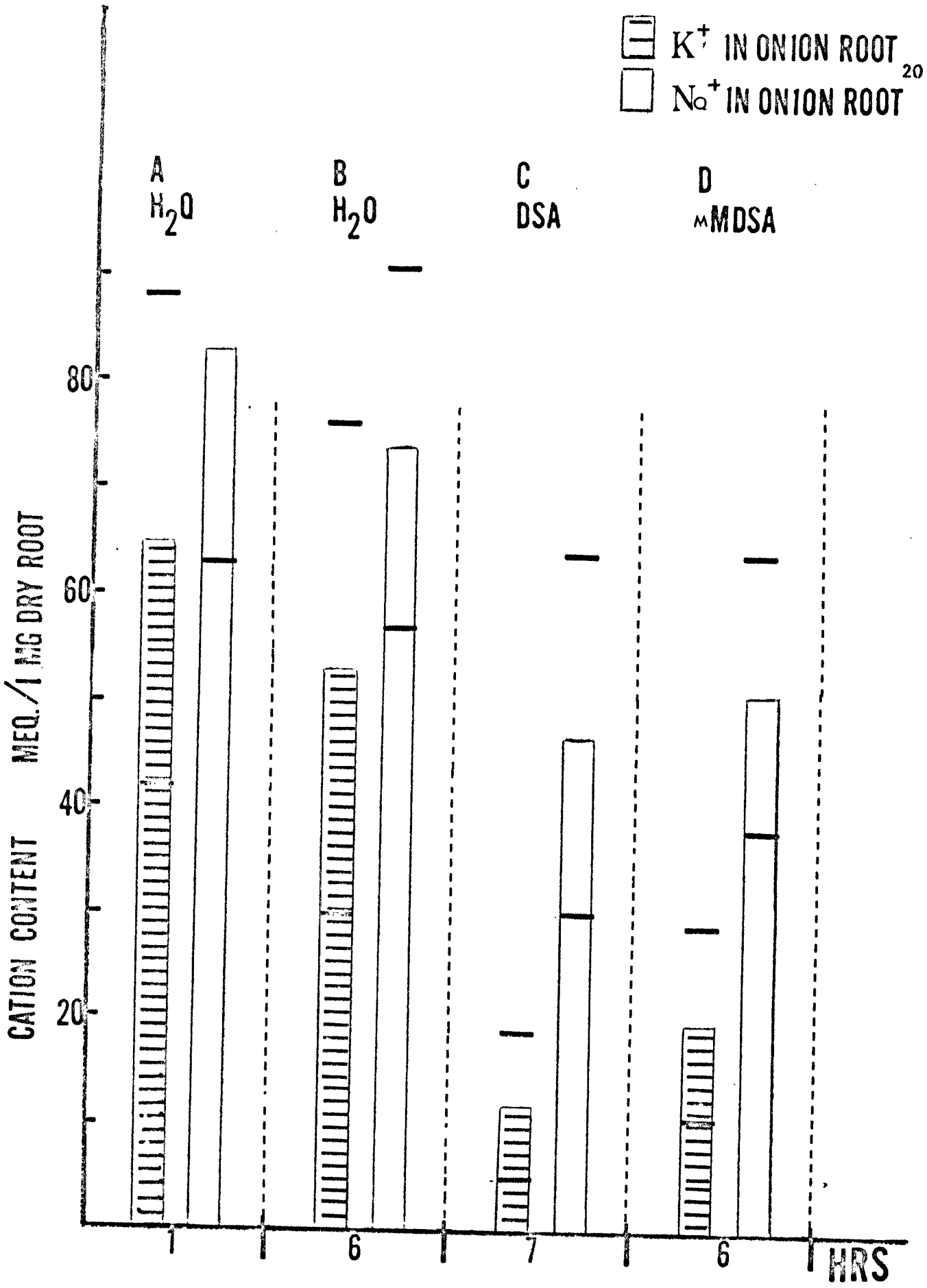

FIG.10 CATION CONTENT OF ONION ROOTS AFTER HOURS IN WATER OR SOLUTION

Summarily, the data clearly indicate a significant loss in both K (at the 0.001 level) and Na (at the 0.01 level) from the roots exposed to both n-DSA and m-MDSA.

Moreover, the data indicate a slight differential effect of n-DSA and m-MDSA on ion flux in isolated roots, the former substance causing a significant loss in K and Na from both the cut basal end and the root surface while the effect of the latter substance appeared to be exerted mainly at the root surface. The absence of a significant increase in Na content in the well solution that appears to counter the loss in Na observed in the ashed roots treated with n-DSA and m-MDSA may be attributed to the short-comings of the instrument used in the Na determination or to the experimenter.

Table I. A Summary of the Effects of n-DSA and Its Mono-methyl Ester on K and Na Flux in Isolated Roots.

A. Changes in the effluent solutions

Experiments	# Roots	Expo. Time	Mean K (meq/1) Control @	Test @	%Change+	Mean Na (meq/1) Control @	Test @	%Change
Water Only	8	-	19.63± 5.2	13.38± 2.4	-32*	15.38± 6.7	10.00± 3.0	-35
n-DSA in H_2O	8	3	16.50± 4.4	24.13± 5.0	+46*	12.13± 4.4	15.00± 7.5	+24
m-MDSA in 1% ETOH	8	2	14.50± 4.6	14.13± 3.6	- 3	23.13± 9.0	20.00± 7.7	-14

B. Changes in the well solutions

			Control	Test	%Change	Control	Test	%Change
Water Only	8	-	1.00± 0.0	1.00± 0.0	+0	7.25± 2.2	8.25± 3.3	+14
n-DSA in H_2O	8	3	1.00± 0.0	1.75± 0.6	+75*	7.38± 2.4	10.63± 3.3	+44
m-MDSA in 1% ETOH	8	2	1.88± 0.7	3.13± 0.7	+66*	13.00± 3.0	15.00± 4.1	+15

C. Changes in root ion contents (Roots ashed following a 2 hours recovery period)

			Mean K (meq/ 1 mg root) T_o	T_6	%Change	Mean Na (meq/ 1 mg root) T_o	T_6	%Change
Water Only	8	-	65.38± 21.9	50.94± 24.8	-22	82.69± 19.1	73.94± 16.5	-11
n-DSA in H_2O	8	3	65.38± 21.9	12.06± 6.9	-82***	82.69± 19.1	47.25± 17.3	-43**
m-MDSA in 1% ETOH	8	2	65.38± 21.9	20.13± 9.0	-69***	82.69± 19.1	50.94± 12.4	-38**

+ $\%\text{Change} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$

@ 95% Confidence Limit ($t_{0.05} \times s_{\bar{x}}$)

* = Significant at 0.05 level

** = Significant at 0.01 level

*** = Significant at 0.001 level

CHAPTER IV

DISCUSSION

In assessing the data in the foregoing experiments, some statements should be made regarding the nature of the test agents used. The n-DSA and m-MDSA obtained from the Humphrey Chemical Co. were not without impurities and could not be crystallized in the laboratory. Moreover, both substances were completely insoluble, regardless of the pH, in one-quarter and/or one-half strength Hoagland's solution. The use of water and 1 per cent ethanol was therefore mandatory. Immediately, the use of a " non-physiological" solution, especially in the control and recovery periods in all of the experiments, may be questionable. Moreover, one may speculate on whether the results obtained here might have been different if the flux of water and ions were measured in roots bathing in a physiological salt solution (Hoagland's). Counting this argument, however, the results obtained in the presence of n-DSA and m-MDSA in water and ethanol were apparently due to the presence of these substances alone and not some complexed test agent resulting from carrier-agent interaction. Moreover, since the data were found to be in overall agreement with those of other workers concerning the effects of n-DSA in particular, the use of distilled water appeared to be valid. To the knowledge of this investigator, no work on root permeability has been reported using m-MDSA. It was difficult to compare the data presented by those of Kuiper (4) and Kramer (9) regarding the effects of n-DSA on root permeability since the

latter workers applied an external suction to their roots. No external suction was applied to the roots in this study in order to study efflux in the absence of an external force similar to transpiration.

Water Efflux

That penetration of n-DSA into the root may be necessary for its action on water efflux was indicated by the fact that it required over one hour of exposure before an effect was noted. Once inside, the n-DSA brought about an increase in water even after the n-DSA solution was replaced with distilled water during the recovery period. On the other hand, exposure to n-DSA for 3 hours resulted in a decrease in water efflux in the roots during the recovery period, indicating a reversible effect. Root exposure to n-DSA for one hour did not result in such a decrease during the recovery period and following the stimulatory phase.

The effects of m-MDSA on water efflux were strikingly different from those observed with n-DSA, although the net effect (stimulation) was the same. Penetration of m-MDSA in both water and ethanol appeared to have occurred more rapidly, resulting in a more rapid, though less prolonged, stimulatory effect than that observed with n-DSA. Moreover, no significant recovery was observed in any of the m-MDSA treated roots. These findings support Kuiper's statement concerning the importance of the chemical structure of n-DSA and its derivatives in bringing about changes in the lipo-protein moiety of membrane structures. It is difficult at this time to account for the differences in the action of n-DSA and m-MDSA on the basis of the data obtained. It would appear that m-MDSA was more effective in regard to the speed and amplitude of the stimulatory phase. That its effect was non-metabolic in nature was indicated by the irreversibility

of the inhibitory phase that followed the relatively short-lived stimulatory phase. On the other hand, the roots exposed to n-DSA did show recovery in water efflux which indicates that the stimulatory effect may, in part, be due to a metabolic action rather than a "simple" physical change in the membrane(s) involved. Moreover, possible metabolic effects of n-DSA on root tissues have been cited by other workers who have reported n-DSA effects on respiration (Lott, J.R., unpublished) and have compared n-DSA effects with those of various metabolic inhibitors (9).

Summarily, the data presented on water efflux are inconclusive in regard to the mode of action (metabolic versus physical) of these two substances and to the possible sites (3) of action (epidermal - cortical - endodermal).

Ion Flux

The finding that n-DSA bring about a leakage of both Na and K from the roots confirmed the data of Kramer (9). The loss of K appeared to have been greater than Na, as expected. Moreover, the loss of K occurred at both the cut basal end as well as from the surface of the n-DSA treated roots. The loss of Na in n-DSA treated roots appeared to have occurred mainly from the root surface rather than from the cut basal end. The m-MDSA treated roots exhibited a loss of K mainly at the surfaces of the roots and not at the cut basal end, once again indicating a pretty sharp difference in the effects of the two substances tested. Davson (2) considers two processes are involved on salt transport of plant roots. They are: (1) the absorption of salts by the epidermal tissues or the root hairs, and (2) the excretion of salts from cortex into the xylem. The first process has been quite

closely linked to aerobic metabolism since removal of oxygen from the roots causes a cessation of absorption and in most cases the salts actually start to flow out of the tissue in the medium. The formation of sap, subsequently, is reduced for a time, yet still occurs at a diminished rate. On the other hand, bleeding under these conditions may be passive resulting from the loss of K from the tissue cells into the xylem, a loss that would increase the osmotic gradient between the medium and the sap, e.g. in cyanide poisoning, the sap of roots contain high concentrations of K indicating a decrease in the cell K content. Since the surrounding media was distilled water in the n-DSA experiments, the extrusion of K in the xylem and root surfaces indicate a breakdown in either the metabolic integrity of the metabolic transport mechanism(s) involved with K intake or in the physical integrity of the membrane per se. The fact that the n-DSA effect on K loss was reversible would tend to support a metabolic effect on the K transport alternation in the membranes. In the case of m-MDSA, K, and less significantly, Na loss appears to be the result of action on the absorption process along the root axis rather than on the exudative phase since the K content of the effluent was relatively constant. On the other hand, the K content of the surrounding media increased during the test period. Again, the m-MDSA effect appeared to be reversible which would indicate some kind of metabolic action on K intake mechanisms. Since the changes observed in the Na content of the effluent and the well solution were statistically insignificant, it is difficult to make valid statements on the effect of n-DSA and m-MDSA on Na transport. The decrease in Na content of the ashed roots following treatment with n-DSA and m-MDSA was statistically significant and therefore indicated

an Na effect by both substances, i.e., both brought about a loss of Na from the roots. Since Na movements in many tissue has been shown to be involved with the flux of K, the results on K flux of this study may have been indirectly due to the effects of the substances tested on the Na flux mechanisms.

Although the net effects of n-DSA and its methyl ester on water and cation flux appeared to be similar in direction, id est, an increase in both water and cation loss, one cannot conclude that the cation flux was brought about by the increase in water efflux or vice versa. In the control experiments, the water efflux was relatively constant for a period of 5 hours. The loss of cations from the control roots also appeared to be constant over even a longer period. However, roots exposed to n-DSA for one hour or 3 hours showed a delayed increase in water efflux, whereas, the cation loss in the same time period was relatively high. The difference in the two responses were not so striking in roots treated with m-MDSA. During the 2 hours exposure, the rate of water efflux was high as was the cation loss. However, in the recovery period, when water efflux was at its lowest rate, the cation flux returned to control values. These findings indicate that water and salt flux in isolated roots are under different control mechanisms, both of which are effected by n-DSA and m-MDSA.

Overbeck and Blondeau (10) proposed that some phytotoxic hydrocarbons tend to separate the lipid layers of the membrane(s), thereby opening up the pores to water. The data regarding the effects of n-DSA water efflux may be explained on this basis; however, the abrupt and sustained decrease in water efflux in the observed m-MDSA treated roots, following the initial

stimulatory phase, could not be accounted for on the same basis. Moreover, it would be difficult to explain the recovery observed in the n-DSA treated roots on the basis of a "lipo-pore" action proposed by Kuiper (4). He suggested that the alkenylsuccinic acids increase permeability of all membranes to water by incorporation of the molecules into the lipid layer of the membranes. Moreover, the increase in permeability with increasing number of CH_2 groups was probably due to greater lipid solubility of the compounds. If n-DSA and/or m-MDSA do act in this manner, the recovery noted in roots treated with these substances may indicate an "active" reversal of the effect, or at least some kind of compensatory action in the tissues.

Summarily, the data indicate clearly that (1) n-DSA and its monomethyl ester bring about significant alternations in the permeability of isolated roots in the absence of transpiration and artificial suction; (2) although the net effect of both these substances on water and cation efflux are similar, they appear to differ in their sites of action (cut basal end bleeding versus loss from the root surfaces); (3) potassium flux is more significantly effected by n-DSA and m-MDSA than sodium flux; (4) the effects on water efflux are not unequivally related to the effects on Na and K flux; and (5) n-DSA and m-MDSA appear to bring about their action on cell loci and/or processes other than the cell membrane and its lipoidal components.

CHAPTER V

SUMMARY

A micropotometric method was described and used to determine, simultaneously, the rates of water and ion efflux from excised onion roots. The ions studied were potassium and sodium, using a flame photometer. The effects of 10^{-3} M solutions of n-DSA and m-MDSA on the two processes were studied.

n-DSA and m-MDSA brought about significant alternations in the permeability of isolated roots in the absence of transpiration and artificial suction. Although the net effect of both these substances on water and cation efflux were similar, they appeared to differ in their sites of action. Potassium flux was more significantly effected by n-DSA and m-MDSA than sodium flux.

n-DSA and m-MDSA appeared to bring about their action on cell loci and/or processes other than the cell membrane and its lipoidal components.

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