Preimaginal lethality after treatment of larval populations of *D. melanogaster* and *D. simulans* with acridine orange

Species	Concentration (mM)	No. flies emerged Males	Females	Total	Sex-ratio (♀ : ♂)	Lethality (%) Observed	Induced*
D. melanogaster	Control	865	822	1687	0.95	15.67	-
	0.1	568	618	1186	1.08	40.70	29.68
	0.5	210	209	419	0.99	79.05	75.16
	1.0	64	55	119	0.85	94.05	92.94
D. simulans	Control	726	770	1496	1.06	25.20	-
	0.1	623	621	1244	0.99	37.80	16.84
	0.5	365	365	730	1.00	63.50	51.20
	1.0	289	291	580	1.01	71.00	61.23

*Corrected for the spontaneous lethality. - For each treatment 2000 eggs were scored.

finding cannot be generalized, since in a previous experiment[13] we reported that *D. melanogaster* was more sensitive to ethidium bromide than *D. simulans* after treatment of adults. Thus, the concentration-resistance pattern may differ considerably, depending on the chemical tested and on the population analyzed.

The essence of this paper can be summarized as follows: acridine orange is toxic for *D. melanogaster* and for *D. simulans*, and the sensitivity of the two species is not the same. More experiments on physiological and genetic hazards of AO on these species are in progress.

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Modulation of membrane permeability by amino acids in *Vinca* petals

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Summary. Exogenously supplied l-amino acids, including proline, differentially reduce the permeability of *Vinca* petal membranes to acid and alkali. It is suggested that these amino acids, which accumulate naturally under drought conditions, may help to sustain drought effects by stabilizing the membranes.

It is well known that free amino acids accumulate under water stress conditions[1]. Enormous accumulation of proline has been repeatedly shown during water stress[2], as well as salt and temperature stress[3]. Proline also accumulates during starvation and mineral deficiency[4]. Various roles have been assigned to this accumulating pool of amino acids, especially proline, including that of nitrogen storage under stress conditions and as an osmoregulator to overcome stress effects[5]. Proline has also been shown to delay wilting and maintain a higher relative water content in wheat and barley seedlings[6]. In an attempt to explore the positive role of these accumulating amino acids in sustaining drought effects, we have investigated the effects of exogenously supplied amino acids on the permeability of membranes in *Vinca major* petals.

Vinca major petals were collected from a wild population and infiltrated with different concentrations of amino acids for 24 h. Amino acids were supplied as the HCl salt in aqueous solutions. Permeability of membranes to acid and base was measured by noting the time taken by 0.5 N HCl or NaOH to pass into the petal[7]. Acid changed the color of the petal from purple to red and alkali from purple to yellow. Though change of color took some time, the time noted is the one for complete change of color. Experiments were conducted in 10 sets in triplicate.

It is clear that different amino acids affect membrane permeability differentially (table 1). L-Proline, L-leucine, L-asparagine, L-arginine and L-alanine double the time taken for alkali to pass through the membrane, and thus reduce the permeability to about half. Similarly, L-phenyl-

Table 1. Effect of amino acids infiltrated into *Vinca* petals on their membrane permeability to alkali (0.5 N NaOH) at room temperature (20 °C). Data expressed as mean time (min) taken to change color or petal from purple to yellow ± SD

Amino acid	Concentration 0	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M
L-Proline	56±1	73±2	84±1	94±3	104±2	111±1	111±3
L-Phenylalanine	56±1	58±2	64±2	75±3	78±1	83±2	82±1
L-Arginine	56±1	63±2	75±2	88±2	97±1	104±2	103±1
L-Leucine	56±1	66±2	78±1	88±2	97±2	107±2	107±1
L-Aspartic acid	56±1	56±2	56±3	54±2	56±2	*	*
L-Asparagine	56±1	60±3	75±3	88±4	96±2	104±2	103±1
γ-Amino butyric acid	56±1	58±2	64±2	77±3	84±2	87±2	87±1
L-Serine	56±1	54±2	56±3	57±2	57±3	68±1	68±1
Glycine	56±1	58±1	57±1	54±1	57±3	56±1	57±1
L-Glutamine	56±1	56±2	64±1	71±2	84±1	96±2	96±2
L-Lysine	56±1	54±2	56±2	67±1	77±1	89±3	89±3
L-Glutamic acid	56±1	57±1	57±1	58±3	58±1	*	*
L-Histidine	56±1	55±2	56±1	56±2	57±1	60±3	61±3
L-Threonine	56±1	57±1	62±2	74±1	86±1	94±2	94±1
L-Alanine	56±1	57±1	69±1	84±1	96±2	108±2	108±1

*Membrane disrupted and pigments leached out.

Table 2. Effect of amino acids infiltrated into *Vinca* petals on their membrane permeability to acid (0.5 N HCl) at room temperature (20 °C). Data expressed as mean time (min) taken to change color of petals from purple to red ± SD

Amino acid	Concentration 0	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M
L-Proline	51±1	67±2	84±2	95±2	97±2	106±1	106±1
L-Phenylalanine	51±1	52±1	57±1	67±2	71±2	77±2	77±1
L-Arginine	51±1	54±2	65±1	74±4	86±2	96±2	97±1
L-Leucine	51±1	64±2	74±2	89±3	94±2	104±2	103±2
L-Aspartic acid	51±1	51±2	50±1	52±2	51±2	*	*
L-Asparagine	51±1	57±2	68±2	85±3	94±1	97±1	97±1
γ-Amino butyric acid	51±1	54±2	60±2	69±2	77±1	84±1	82±1
L-Serine	51±1	53±1	53±2	55±2	54±2	60±2	61±1
Glycine	51±1	53±1	53±1	55±2	54±3	53±2	53±1
L-Glutamine	51±1	52±1	52±1	60±3	78±2	88±1	88±2
L-Lysine	51±1	56±2	58±2	64±3	77±3	85±2	85±2
L-Glutamic acid	51±1	52±1	51±1	52±2	51±2	*	*
L-Histidine	51±1	54±2	53±1	53±1	52±1	54±2	54±2
L-Threonine	51±1	52±1	54±2	63±4	74±1	84±2	85±3
L-Alanine	51±1	64±1	74±1	86±1	94±1	104±1	104±1

*Membranes disrupted and pigments leached out.

alanine, γ-amino butyric acid, L-glutamine and L-threonine reduce permeability considerably. Glycine, L-aspartic acid and L-glutamic acid show no effect, while with L-serine, L-histidine and L-lysine at lower concentrations (10^{-6}–10^{-4} M) no effect was observed but at higher levels of 10^{-3}–10^{-1} M a noticeable reduction in permeability to alkali was clear. For all amino acid showing an effect, the effect increased with increase in concentration of amino acid. An almost similar pattern of permeability was also observed with respect to acid (table 2). Proline, L-leucine, L-asparagine, L-arginine and L-alanine reduced the permeability to half; L-phenylalanine, L-glutamine, L-lysine and L-threonine significantly reduced the permeability while L-aspartic acid, L-serine, glycine, L-glutamic acid and L-histidine showed no effect. The dicarboxylic amino acids, i.e. L-aspartic acid and L-glutamic acid at 10^{-2} and 10^{-1} M damage the membrane and pigment leaches out.

Looking at the fact that with increasing amino acid concentrations, permeability is progressively decreased, it appeared that these amino acids might be blocking the surface of the membranes in some manner, thus causing decreased permeability. To check this further, another experiment was set up where amino acids were not infiltrated into the tissue, but were provided with the acid/alkali in the outer medium. Even then the permeability of the membranes was decreased to almost the same extent (table 3), showing that this effect is at the membrane surface.

Thus it is clear that metabolites accumulating during water-stress, especially amino acids, have a direct effect on membrane permeability and stabilization. Further, it is emphasized that besides proline other amino acids also play an important role in membrane stabilization. Naik and

Table 3. Effect of amino acids (10^{-1} M) supplied in the ambient medium with acid (0.5 N HCl) or alkali (0.5 N NaOH), on the membrane permeability of *Vinca* petals at room temperature (18 °C). Data expressed as mean time (min) taken to change color of the petal ± SD

Amino acid (10^{-1} M)	+ Acid (0.5 N)	+ Alkali (0.5 N)
Water (control)	53±1	59±2
L-Proline	106±1	110±3
L-Phenylalanine	77±1	82±1
L-Arginine	96±1	104±1
L-Leucine	104±1	107±2
L-Aspartic acid	51±1	56±1
L-Asparagine	97±1	104±1
γ-Amino butyric acid	82±1	88±1
L-Serine	62±1	68±1
Glycine	53±1	57±1
L-Glutamine	88±1	97±1
L-Lysine	85±1	95±1
L-Glutamic acid	52±1	58±1
L-Histidine	55±2	63±2
L-Threonine	87±2	97±1
L-Alanine	104±1	109±1

Srivastava[8] have earlier shown that di- and polyamines such as agmatine, putrescine, cadaverine, spermidine and spermine significantly reduced pigment efflux from beet root discs, by reducing membrane permeability, and suggested that this stabilizing effect on beet root membrane is due to their binding to phospholipid components of the membrane. The case with amino acids may be similar. Proline has been shown to act as a cryoprotectant in maize and potato protoplast cultures by stabilizing the membrane; besides proline, γ-amino butyric acid, hydroxyproline and aspartate also helped in cryoprotection of the maize protoplast[9]. Altman et al.[10], studying stabilization of oat leaf protoplasts, also showed that L-arginine and L-lysine, besides other polyamines, inhibited their senescence by stabilizing the protoplast membrane.

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Biochemical studies of CELO virus: an oncogenic avian adenovirus[1]

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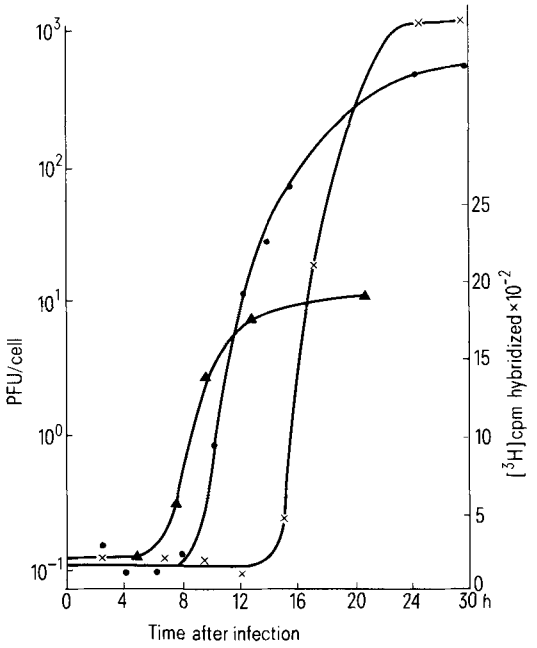
Summary. The onset of viral mRNA and DNA synthesis in CELO virus infected cells occurred about 5 and 8 h respectively after infection. Viral maturation occurred approximately 6 h after DNA synthesis at 14 h post infection.

Chick embryo lethal orphan (CELO) virus is a DNA containing avian adenovirus[2,3]. Its oncogenic potential was established by inoculation of CELO virus into non-permissive hosts[4,5] and in tissue culture cells[6,7]. Moreover, fragment mapping of restriction enzyme digests of CELO virus DNA[8] and sequences of viral DNA found in transformed cells[9] have been reported. However, the biochemical events associated with macromolecular syntheses of the viral multiplication cycle have not been published. The events reported here are concerned with the time of viral mRNA and DNA synthesis and the time of viral maturation occurring in chick embryo kidney tissue culture cells infected with CELO virus.

DNA-RNA hybridization was employed to determine the time of viral mRNA synthesis. CELO virus was inoculated into embryonated 11-day-old chicken eggs and the allantoic fluid was harvested at time of embryo death. The virus was purified by isopycnic CsCl gradient centrifugation[2,10], and DNA was extracted from the virus by digestion with pronase B (100 µg/ml) and 1% sodium dodecyl sulfate for 30 min followed by purification with a phenol, chloroform, and isoamyl alcohol mix (25:24:1)[2,11]. After denaturation, DNA was immobilized onto 6.5-mm nitrocellulose filters (1 µg/filter)[12]. Cells for tissue culture and virus infection were obtained from the kidneys of 19-day embryos. They were minced and trypsinized (0.1% trypsin and 0.05% EDTA). Cells were grown in MEM Eagles with Earle's salts supplemented with 10% tryptose phosphate broth (TPB) and 10% calf serum (CS). Monolayers were established after 48 h of growth and used for CELO virus infection at 5 PFU/cell (see below). At various times after infection, up



Macromolecular events during CELO virus infection of chick embryo kidney tissue culture cells. Time of viral mRNA synthesis (▲—▲), viral DNA replication (●—●) and viral maturation (×—×) are indicated at various times after infection. Viral mRNA synthesis was determined by DNA-RNA hybridization and viral DNA and viral maturation were determined by plaque titration.